ALUMINUM EFFECT ON GROWTH OF CITRUS ROOTS IN SOLUTION AND SOIL SYSTEMS

by

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Aluminum phytotoxicity may be a growth-limiting factor for citrus roots growing in acid soils. Four experiments were conducted to investigate the effects of Al on citrus root growth in solution and soil systems.

In a laboratory study, two sets of supernatant nutrient solutions were prepared and evaluated for Al phytotoxicity studies. For the pH 4.0 and pH 4.5 sets, actual Al concentrations ranged from 0.1 to 171 mg Al L^{-1} and from 0.1 to 10 mg Al L^{-1}, and P concentrations were about 1 mg P L^{-1} and 0.2 mg P L^{-1}, respectively.

In a greenhouse study, five 6-month-old citrus rootstock seedlings were grown in supernatant nutrient solutions containing seven levels of Al at pH 4.0 for 60 days. According to the response of new-growth fresh weight of whole plants to Al concentrations in solution, relative Al-tolerances were Cleopatra mandarin (C. reshni Hort. ex Tan.) > Rough lemon (C. jambhiri Lush.) = Sour orange

(*C. aurantium* L.) > Swingle citrumelo (*C. paradisi* x *P. trifoliata*) > Carrizo citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.]. The critical Al concentrations in solution for toxic effects were 12.2, 5.1, 5.1, 4.5, and 1.8 mg Al L^{-1}, respectively, for the above rootstocks. Concentrations below or above the critical Al levels caused either beneficial or toxic effects, respectively. When Al concentrations in nutrient solution increased from 0.1 to 4.8 mg Al L^{-1}, Al, K, Mg, and P concentrations in roots and Al, K, and P levels in shoots increased; whereas Ca, Zn, Cu, Mn, and Fe in roots and Ca, Mg, Cu, and Fe in shoots decreased. Aluminum-tolerant rootstocks accumulated more Al in their roots than did Al-sensitive rootstocks. The more Al-tolerant rootstocks contained higher Fe concentrations in their roots than did the less tolerant ones when Al concentrations in solution were lower than 8.3 mg Al L^{-1}.

In a field experiment, E-horizon soil was treated with either lime or four levels of Al, placed in porous bags, and then implanted in the surface horizon of a citrus grove for 46 days. Results indicated that the critical Al concentration for toxicity in the saturation extract of soils was 23 mg Al L^{-1} for root growth of mature trees of Sour orange rootstock.

In another similar field experiment, Bh-horizon soil was amended with either lime or phosphogypsum, implanted, and collected after 55, 84, 113, and 139 days. Application of lime significantly increased fibrous citrus-root growth while phosphogypsum did not. The soil amended with phosphogypsum had a lower pH, higher salinity and exchangeable Al; higher Ca^{2+} and Mg^{2+} and lower P ($H_2PO_4^-$ and HPO_4^{2-}) and Cl^- contents in the saturation extract than the non-amended soil.

CHAPTER I

GENERAL INTRODUCTION

Aluminum toxicity is probably the most important growth-limiting factor for plants in most strongly acid soils. A number of crops have been studied with respect to their response to Al phytotoxicity. Citrus grows widely in tropical and subtropical areas in which soils are highly weathered and generally acidic. Aluminum toxicity may be an important factor limiting citrus growth in these acid soils.

Few studies have been conducted on the effects of Al on citrus growth (Haas, 1936; Liebig et al., 1942; Yokomizo and Ishihara, 1973; Worku et al., 1982). These researchers found that low Al concentrations in solution stimulated, but high concentrations depressed, root growth of some citrus species. However, no experimental results have been reported in the literature for screening and evaluation of citrus rootstocks for Al tolerance. The Al phytotoxicity levels are still not known for many citrus rootstocks, and few data exist on the effects of Al on the mineral nutrition of citrus.

Solution culture has been frequently used for Al phytotoxicity studies. Aluminum effects on roots are confounded by many factors, such as pH, temperature, concentrations of P, Ca, and Mg (Rhue and Grogan, 1977). The common problems in the previous studies with nutrient solution were the confusion of added levels of Al with actual concentrations of Al in the nutrient solution, and the confounding effects of P (Bollard, 1983; Marschner, 1986).

Research on the effects of Al on citrus growth has been mainly limited to nutrient-solution studies. Field studies are highly desirable to evaluate Al effects on citrus root growth under field conditions.

In Florida, Spodosols have been increasingly used for citrus production. The spodic horizons of these soils are generally very acid and have high Al contents (Myhre et al., 1987), so it is probable that Al toxicity problems occurs in these subsoils. Perhaps it is worthwhile to determine whether phosphogypsum could be used as an ameliorant for the subsoil acidity syndrome.

The overall objectives of this dissertation research were to develop a better understanding of Al effects on citrus growth and nutrient uptake, both in nutrient solution and under field conditions, and to evaluate citrus rootstocks used in Florida for Al-tolerance. In addition, lime and phosphogypsum were tested as an ameliorant for the acidity syndrome of spodic horizon soils.

This dissertation is divided into six parts. Chapter II is a review of the literature for the entire set of studies. Chapter III describes a supernatant solution containing various levels of Al and similar concentrations of P that was prepared as a culture solution for Al studies. In Chapter IV, five rootstocks were studied in nutrient solution for their Al-tolerance, and their elemental composition of roots and shoots as affected by Al concentrations. In Chapter V, a field experiment was conducted using the implanted soil-mass technique to evaluate the critical Al concentration in saturation extracts of soils for toxicity and elemental compositions in roots as affected by Al concentration under field conditions.

In Chapter VI, the effects of lime and phosphogypsum on fibrous citrus-root growth and properties of Bh horizon soil were studied in the field using the implanted soil-mass technique. Finally, in Chapter VII, the studies are summarized and recommendations are provided for further work.

CHAPTER II

LITERATURE REVIEW

Beneficial Effects of Aluminum on Plant Growth

Aluminum is not regarded as an essential nutrient, but low concentrations can sometime increase plant growth or produce other desirable effects. An early report of the stimulation of plant growth was made by Mazé (1915), and similar reports have continued to appear from a number of laboratories. Plants that have shown positive growth response to Al include rice, maize inbreds, eucalyptus, tea, peach, sugar beet, tropical legumes, wheat, and pea. For a more-thorough review on this subject, see Bollard (1983) and Foy (1984). The growth stimulus is greater for Al-tolerant cultivars than for Al-sensitive cultivars (Howeler and Cadavid, 1976; Clark, 1977). The stimulating Al concentrations are usually about 1 mg L^{-1} or less. In the tea plant, however, growth stimulation is observed at Al concentrations as high as 27 mg L^{-1}.

The mechanisms of Al beneficial effects are debatable and may be different for different plant genotypes and growth media. Possible explanations (Foy, 1984) include (1) increasing P, Fe, and Ca uptake; (2) preventing toxicities of Cu, Mn, and P; (3) altering the distribution of growth regulators; and (4) serving as a fungicide.

The beneficial effects are the exception, however, and toxic effects of Al on plant growth in soils of low pH are the rule.

Phytotoxicity of Aluminum

Aluminum toxicity is probably the most important growth-limiting factor for plants in most strongly acid soils and mine spoils (Foy, 1974; McLean, 1976).

The symptoms of Al toxicity are not easily identified. Aluminum toxicity effects are first detected in the root system, in which there is reduced growth of the main axis, resulting in short thick roots, and in the inhibition of lateral root formation (Alam and Adams, 1979; Bollard, 1983). In plant tops restricted growth is often the main symptom of toxicity, but sometimes mottling and necrotic symptoms can appear on leaves as well (Cate and Sukhai, 1964). In some plants the foliar symptoms resemble those of P deficiency. In others, Al toxicity appears as an induced Ca or Fe deficiency (Foy, 1984). Young seedlings are generally more susceptible to Al toxicity than are old plants (Thaworuwong and Van Diest, 1974).

Several distinct positive modes of Al-toxic mechanisms have been investigated. Excess Al has been reported to interfere with cell division in root tips and lateral roots; increase cell-wall rigidity by cross-linking pectins; reduce DNA replication by increasing the rigidity of the DNA double helix; fix P in less-available forms in soils and on root surfaces; decrease root respiration; interfere with enzymes governing sugar phosphorylation and the deposition of cell wall polysaccharides; and interfere with the uptake, transport, and use of several essential nutrient elements, including Ca, Mg, K, P, and Fe (Foy, 1984).

Excess Al may reduce the uptake of certain essential elements and increase that of others (Ali, 1973; Alam, 1981; Duncan et al., 1980). Aluminum toxicity is often associated with Al-induced P toxicity (McCormick and Borden, 1972) or Al-induced P deficiency (James et al., 1978). Aluminum-induced Fe deficiency is frequently mentioned in the literature (Alam, 1981; Clark et al., 1981), and aluminum x Ca interactions are important in acid soils. Lance and Pearson (1969) showed that reduced Ca uptake was the first externally observed symptoms of Al damage on cotton seedling roots. Lund (1970) found that Ca reduced the detrimental effects of Al in nutrient solution. However, the data for effects on nutrient uptake are difficult to interpret in terms of Al toxicity mechanisms. No one pattern of elemental accumulation applies to all cases of Al injury (Foy, 1984), with the entire array of elements in the tops of Al-injured plants probably representing the accumulated systematic effects of initial root injury by Al. Such effects are generally too far removed from the initial root injury to reveal Al-toxicity mechanisms (Foy, 1984). The reduction in levels of some elements is also a result of reduced root surface area rather than a specific effect of Al (Clarkson, 1966).

Differential Aluminum Tolerance of Plants

Different plant species and varieties differ widely in their tolerance to excess Al in the growth medium. There is now considerable activity devoted to breeding crop cultivars better adapted to acid-soil conditions, with work in this field having resulted in the detection of certain differences between susceptible and tolerant cultivars. However, the exact physiological mechanisms of Al

tolerance are still being debated; tolerance may be controlled by different genes, acting through different biochemical pathways in different plants (Foy, 1984).

Three major mechanisms are involved in Al tolerance: (1) exclusion from uptake (excluder plants); (2) inactivation in the roots (excluder, includer plants); and (3) accumulation in the shoots (includer plants). Mechanism (3) exists mainly in highly Al tolerant species of natural vegetation, with only a few cultivated species being Al includers. In crop species, mechanisms (1) and (2) predominate, and it is often difficult to differentiate between the two (Marschner, 1986) The following factors may be of primary importance in the exclusion mechanism:

1. Rhizosphere pH. When Al is present, some tolerant cultivars tend to raise the external pH faster than sensitive cultivars, both in the nutrient solution (Foy et al., 1967) and in the rhizosphere of soil culture (Mugwira and Petel, 1977). A slight pH increase at the root surface or in the free space is probably sufficient to lower the charge of Al, which leads to the formation of Al polymer species. These polymer species may facilitate P uptake.

2. Aluminum uptake and distribution. Some Al-tolerant plants have a lower Al concentration in roots than do Al-sensitive plants. In this case, Al tolerance apparently involves an exclusion mechanism. Other Al-tolerant plants have either more or less Al in their tops than do Al-sensitive plants. Such plants have higher internal tolerance to Al. Tea, certain Hawaiian grasses, pine trees, and mangrove are examples of Al accumulators (Foy et al., 1978), but little is known about the forms in which Al may exist in the foliage

of accumulator species (Bollard, 1983). Some effort has been made to establish critical levels of Al for toxicity in plant tops (Wallace and Romney, 1977; Duncan, 1982).

3. Nutrient uptake. Some Al-tolerant plants are also NH_4^+-tolerant. This NH_4^+-tolerance is important in strongly acid soils, where high concentrations of NH_4^+ may be present (Foy and Fleming, 1982). Aluminum tolerance in certain cultivars of wheat, barley, soybean, and snapbean has been associated with the ability to resist Al-induced Ca deficiency (Foy et al., 1978). In many plants, Al tolerance also appears to be closely related to P-use efficiency (Foy et al., 1978).

4. Organic Al complexes. Naturally occurring organic acids in Al-tolerant species chelate Al and thereby reduce the Al-P precipitation expected at normal pH levels in plant sap (Jones, 1961). Klimashevskii and Chernysheva (1980) found that the roots of Al-tolerant varieties of pea, maize and barley contained substantially higher concentrations of citric acid than did those of Al-sensitive varieties of the same species. Complexation of Al by organic acids not only provides protection against the harmful effects of free Al on root growth, but is also important for the uptake of P (Marschner, 1986).

Study Methods for Al Phytotoxicity

Study methods for Al phytotoxicity normally include solution culture, soil culture in the greenhouse, and field experiments. Solution culture has been used most frequently.

Solution culture (nutrient solution or soil solution) has been used for studying the relationship between Al speciation and

phytotoxicity. Attempts have been made to relate the concentrations of Al species in solution (Al^{3+}, hydroxy-Al monomers, hydroxy-Al polymers, $AlSO_4^+$, et al.) as obtained by calculation with GEOCHEM or other programs to plant-growth parameters in order to find out what species is most toxic to plant growth. However, there is no clear consensus as to the specie(s) predominantly responsible for phytotoxicity (Blamey et al., 1983; Alva et al., 1986; Parker et al., 1988, 1989).

Rapid screening methods have been developed, mainly in solution culture systems. Most workers have found that the primary damage caused by Al occurs in the roots (Bouma et al., 1981; Bollard, 1983). In most crop species, the relative root length of plants exposed versus those not exposed to Al is the most appropriate parameter (Marschner, 1986). Because Al toxicity to roots is affected by many factors, such as pH and concentrations of P, Ca, and Mg, the biggest problem in developing rapid screening techniques is finding an appropriate combination of these factors to use (Rhue and Grogan, 1977). In some instances the classification of genotypes based on their Al tolerance via the rapid screening methods correlates well with the growth response of these genotypes in acid soils (Howeler and Cadavid, 1976). However, the correlations often are quite poor (Nelson, 1983). These discrepancies are not surprising and indicate that a) factors such as rhizosphere pH have been insufficiently considered, and b) factors other than excessive Al levels may have been involved and may have had an even more harmful effect on growth.

There has been much work with plants grown in nutrient solution, on the effects of Al concentration and possible modifying factors on

the growth and elemental composition of plants. There are considerable technical difficulties with such experiments. There is often confusion about what level of Al is actually present in solution. A general problem in most studies on the beneficial effects of Al on plants is the contamination of the nutrient solution with Al. Reasonably high levels are frequently reported in plants growing in conventional culture solutions without any added Al (Wilkinson and Gross, 1967). Moreover, the reduced solubility of aluminum phosphate with increasing pH greatly restricts the combinations of Al and phosphate concentrations and pH of culture solution which can be compared. The other problem in some studies is the confounding effects of P and Al. When zero or small amounts of Al are added, excessive P levels are quite common (Marschner, 1986). When large amounts of Al are added, P is often deficient in the nutrient solution due to the precipitation of aluminum phosphate.

Field experiments are very important in Al-phytotoxicity and screening studies for Al tolerance (Foy et al., 1974; Mugwira et al., 1981). However, field experiments are labor-intensive, require several months or more for completion, and are often influenced by secondary factors such as the variation of soil properties. Application of Al to a large field area is not practical. It also is very difficult to study root systems of crops in field experiments without disturbance of the soils and the root system, particularly for large plants such as trees.

Studies of Aluminum Effects on Citrus

Citrus species are grown widely in tropical and subtropical areas of high annual rainfall in which the soils are almost always

acid. Citrus trees were domesticated from wild ancestors in Eastern and Southern Area (Hill, 1937), where most soils are highly acid. Aluminum phytotoxicity may be an important factor limiting citrus growth in the more acid of these soils. However, only a few studies have been reported in this field.

An early report of Al effects on citrus growth was made by Haas (1936). He used leafy-twig cuttings of some citrus in a nutrient solution and found that low Al concentrations stimulated root growth while high Al concentrations were toxic. He also found that addition of Al increased P uptake. Liebig et al. (1942) made similar findings. They also found that addition of Al reduced Cu toxicity. Yokomizo and Ishihara (1973) conducted a solution culture with a wide range of Al additions. They found that, at low Al additions, citrus root growth increased. At 100 mg Al L^{-1} addition, however, citrus root growth was extremely depressed. Worku et al. (1982) conducted a study with highly weathered Oxisols in Hawaii. They found that high levels of Al and Mn were toxic to some citrus species. The effects of Al and Mn, however, were confounded. Other researchers (Sekiya and Aoba, 1975; Huang, 1983) have linked high Al concentration to poor citrus growth.

Additional information about Al effects on citrus is needed and the following aspects are in particular need of further study:

1. Comparing the effects of Al on several citrus species, to find out the critical Al concentrations for toxicity in solution culture.

2. Screening Al tolerance of citrus species systematically.

3. Study of the relationship between Al effects and macro- and micro-nutrients.

4. Study of the mechanisms of beneficial and toxic effects of Al on citrus.

5. Investigation of the effects of Al on citrus growth in soils, particularly in the field, and assessment of the critical Al concentrations in soil solution or in citrus leaves which reflect toxicity.

6. Testing amendments which may be used practically in citrus groves to ameliorate Al toxicity.

CHAPTER III

SUPERNATANT SOLUTIONS CONTAINING VARIOUS LEVELS OF ALUMINUM AND SIMILAR CONCENTRATIONS OF PHOSPHORUS AS CULTURE SOLUTIONS FOR THE ALUMINUM STUDY

Introduction

Solution culture has been widely used to study the effects of Al concentration on the growth and elemental composition of plants and to screen crop species for Al tolerance. Normally, authors report the amounts of Al added to the solution but not the actual Al concentration in the growth solution. Many nutrient culture studies have employed high Al additions (up to 7.4 mM or more) (Tanaka and Navasero, 1966; Yokomizo and Ishihara, 1973), high P additions (up to 0.48 mM) (Moore et al., 1976; Nelson, 1983; Williams, 1982) and high solution pH (4.8 or higher) (Malavolta et al., 1981; Tanaka and Navasero, 1966; Williams, 1982). These conditions have probably resulted in the precipitation of $Al(OH)_3$ and aluminum phosphate (Blamey et al., 1983; Yokomizo and Ishihara, 1973). Such losses of Al from the test solutions would be expected to cause an overestimation of the threshold concentration for Al toxicity (Asher, 1981). Another general problem in most studies on the effect of low levels of Al on plant growth has been the contamination of the nutrient solution with Al even where the Al solution level was assumed to be zero (Marshner, 1986; Tanaka and Navasero, 1966; Wilkinson and Gross, 1967). Therefore, there are good reasons to report the actual Al concentrations of the growth solution.

The precipitation of aluminum phosphate also causes the decrease of actual P concentration in the solution (Munns, 1965; Tanaka and Navasero, 1986). For a given pH and P addition, if the amount of added Al is zero or small, there may be a P-toxicity problem (Marschner, 1986). If the amount of added Al is large, there may be a P deficiency problem. Either of these problems may confound the effects of Al on plant growth. In many previous studies, beneficial or toxic effects of Al were reported to be related to P (Foy, 1984). Some researchers also found that P toxicity or deficiency affected the Al toxicity symptoms and critical concentrations in solution culture (Tanaka and Navasero, 1966). Therefore, it is necessary to report the actual concentration of P in the growth medium. It is also important to get similar concentrations of P in nutrient solutions with different Al concentrations, although there are considerable technical difficulties because of uncertainty which extends even to the prediction of precipitation.

In nutrient solutions with amorphous precipitates of $Al(OH)_3$, aluminum phosphate, and other compounds, it is difficult to estimate the actual concentrations of Al, P and other elements which may react with Al and P to form precipitates during the growth periods. Such precipitates may become a sink or source for the elements in the solution. The actual concentrations of the elements in the solution may dynamically change as well. Furthermore, with continuous aeration amorphous precipitates may deposit on root surfaces and this coverage may affect the physiological function of the roots. It is preferable to use supernatant solution (filtered or siphoned) instead of turbid solution to grow plants in Al studies.

In earlier work, some techniques were used to avoid precipitation problems. Munns (1965) suggested comparing the effects of Al concentration in culture nutrient solution only at phosphate concentration of 19 μM (0.59 mg P L^{-1}) or less, Al concentrations on the order of 100 μM (2.7 mg Al L^{-1}), and pH values of 4.0-4.2 to avoid precipitation problems. Nonetheless, many experiments have been carried out with treatments exceeding such narrow limits. In order to avoid precipitation of aluminum phosphate, phosphorus has been omitted (Moore et al., 1976), or plant roots have been alternately exposed to culture solutions containing either Al or P, or split-root techniques have been used. These modifications, however, impose their own constraints on the interpretation of experimental results (Pierre et al., 1932; Wright, 1937).

The objectives of this study were two-fold: (1) to investigate the actual concentrations of Al and P in nutrient solution under different pH and different Al and P additions; and (2) to develop and test a supernatant-solution method for Al studies, in which the supernatant solutions contain various levels of Al and similar concentrations of P.

Materials and Methods

General

All reagents were of analytical grade and double-deionized water was used. The basal nutrient solution used for this study contained about one-fourth of the macronutrient concentrations, except for P, of no. 1 Hoagland and Arnon solution (1950). This basal nutrient solution has been used previously for some other Al

studies (MacLeod and Jackson, 1967; Yokomizo and Ishihara, 1973). The basal nutrient solution contained the following elements in mg L^{-1}: 50 N from NH_4NO_3, 50 K from K_2SO_4, 50 Ca from $CaCl_2$, 15 Mg from $MgSO_4 \cdot 7H_2O$, 2.0 Fe from $FeSO_4 \cdot 7H_2O$, 0.2 Mn from $MnSO_4 \cdot H_2O$, 0.1 Zn from $ZnSO_4 \cdot 7H_2O$, 0.02 Cu from $CuSO_4 \cdot 5H_2O$, 0.2 B from H_3BO_3, and 0.02 Mo from $(NH_4)_6MO_7O_{24} \cdot 4H_2O$. Phosphorus and Al were not included in the basal nutrient solution. In preparation of mixed solution aluminum was added from $Al_2(SO_4)_3 \cdot 18H_2O$ and P from $NaH_2PO_4 \cdot H_2O$. The filter paper used was Whatman 42, which was ashless and had a minimum particle-retention diameter of 2.5 µm. The pH of solution was measured using a combination glass electrode, and the electrical conductivity (EC) was measured using a conductivity bridge. Elemental composition of solution was determined by ICAP (Inductively coupled argon plasma) emission spectroscopy. In Experiments 1 and 2, all treatments were replicated three times.

Experiment 1. Effects of pH and Additions of Al and P on EC and Concentrations of Al and P in Filtrated Nutrient Solution

Six levels of Al addition (0, 5, 25, 50, 100, and 500 mg L^{-1}) were used in factorial combination with two levels of P addition (3 and 15 mg L^{-1}) and four pH levels (3.5, 4.0, 4.5 and 4.8). Aluminum or P salt was added separately to each 200 mL of the basal nutrient solution, and dissolved. The solution containing P then was added to the Al solution slowly with vigorous stirring by magnetic stirrer. Then the pH of the mixed solution (400 mL) was adjusted with 0.5 or 1 M first and then with 0.1 M HCl or NaOH. The HCl or NaOH was added to the solution drop by drop, with vigorous stirring by magnetic stirrer. After aging for seven days at 25°C, the solution

was filtered and the filtrate was analyzed for EC and concentrations of Al and P.

In addition, four trials were conducted to investigate a) the composition differences between filtered nutrient solution and supernatant solutions obtained by siphon, b) the effect of equilibration time and temperature, and c) the effect of storage temperature on composition of filtrate.

Experiment 2. Supernatant Solutions Containing Several Levels of Al and Similar Concentrations of P

Two trials were conducted. The first was to develop one set of supernatant solutions which contained various levels of Al (0 to 150 mg L^{-1}), with a similar P concentration (1 mg L^{-1}) and at the same pH (4.0). There were a number of Al-addition levels (0 to 320 mg L^{-1}) in factorial combination with a number of P-addition levels (0 to 320 mg L^{-1}). The pH of all mixed solutions was adjusted to 4.0. The second trial was to develop a second set of supernatant solutions which contained various levels of Al (0 to 10 mg L^{-1}) with similar P concentration (0.2 mg L^{-1}) and at the same pH (4.5). There were a number of Al-addition levels (0 to 160 mg L^{-1}) in factorial combination with a number of P-addition levels (0 to 160 mg L^{-1}). The pH of all mixed solutions was adjusted to 4.5. The procedure of mixing, aging, and filtering were the same as described in Experiment 1. The filtrates were analyzed for EC, and for concentrations of Al, P, and other elements.

Experiment 3. Elemental Composition of Large-volume Supernatant Solutions Prepared Manually in the Greenhouse

In order to make 100 liters of the supernatant solution developed in Experiment 2 as the first set, 55 liters of basal

nutrient solution was prepared in a 120-liter plastic container. Aluminum and P solutions were prepared with water, each in separate 20-liter plastic containers. The aluminum solution was mixed with the basal nutrient solution in a 120-liter container. Then the P solution was added. The solution was mixed by hand-stirring with a plastic bar. The pH was adjusted with additions of 3.5 $\underline{M}$ HCl or NaOH from a wash bottle, and the solution was mixed. The mixed solution was then made up to 100 liters with basal nutrient solution. After the solution was aged for 7 days at room temperature in the greenhouse, the supernatant liquid was siphoned. Electrical conductivity, and Al and P concentrations in the supernatant solution, were determined. The supernatant solution was used for Experiment 4 also.

Experiment 4. Test of Supernatant Solutions as Culture Solutions Using Citrus Seedlings

The objective of this experiment was to use citrus seedlings to test the first set of supernatant solutions developed in Experiment 2 and prepared in Experiment 3.

Five citrus rootstocks were used: Carrizo citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.], Cleopatra mandarin (*C. reshni* Hort. ex Tan.), Rough lemon (*C. jambhiri* Lush.), Sour orange (*C. aurantium* L.), and Swingle citrumelo (*C. paradisi* x *P. trifoliata*). Six-month seedlings (liners) were obtained from nurseries. Uniform seedlings were selected and their roots were thoroughly washed with tap water, and then given a final rinsing with deionized water. The seedlings were transferred to the supernatant solutions prepared in Experiment 3. Ten-liter pails (25 cm diam. x 21 cm height) were used to hold the nutrient solution. Five holes were made in the plywood

lid of the pails, one for each of the five rootstock seedlings. The entire root system of the five seedlings was submerged in the solution, and the solution was continuously aerated. This also served to keep the solution uniformly mixed. Air-conditioning was used in the greenhouse to maintain the air temperature in a range from 25 to 35°C. The pails were put inside pools filled with water which was circulated by a pump and passed through a cooling system. The water temperature in the pools, as well as the solution temperature in the pails, was maintained at 25 ± 1°C.

Seven Al concentrations were used. Eight pails contained the same Al concentration and five rootstock seedlings. A total of 56 pails containing treatment solutions were randomly assigned positions in the pools. The pH levels were checked every 2 to 3 days and adjusted to 4.0 by additions of diluted HCl or NaOH as necessary. The solution level was maintained by addition of deionized water in a quantity sufficient to offset loss due to evapotranspiration every two days. The treatment solutions were renewed every 20 days. The seedlings grew in the solution for 60 days. At the end of the last 20-day growth period, samples of the solutions were taken to determine concentrations of Al, P, and other elements. At the beginning and end of the last 20-day growth period, shoot heights of the seedlings were measured. Photographs were taken at the end of the experiment.

Results and Discussion

Experiment 1. Effects of pH and Additions of Al and P on EC and on Concentrations of Al and P in Filtrated Nutrient Solution

Aluminum concentrations increased with increased levels of Al addition but decreased with increased pH or increased levels of P

addition (Tables 3-1 and 3-2). As pH increased or P addition increased, more precipitate was found in the mixed solution. When pH increased from 3.5 to 4.5, Al concentration decreased drastically, and, when pH increased to 4.8, Al concentration became extremely low, averaging only a few mg Al L^{-1}. Even when 500 mg Al L^{-1} and only 3 mg P L^{-1} were added, the actual Al concentration was only 3.6 mg L^{-1}. With small amounts of Al addition, such as 5 mg Al L^{-1} together with 15 mg P L^{-1}, Al concentration was essentially zero in the filtered nutrient solution. The large difference between the amounts of Al added and actual Al concentrations in the filtered nutrient solution suggests that it is necessary to report the actual Al concentrations in the growth nutrient solution for Al studies.

Phosphorus concentrations increased with increasing levels of P addition, but decreased with increasing pH and addition levels of Al. It was noteworthy that at pH 4.5 or higher, when 25 mg Al L^{-1} or more were added, the P concentration was zero, whether 3 or 15 mg P L^{-1} had been added. These treatments could result in extreme P deficiency, and would confound the Al-toxicity effects. In contrast, when Al addition was zero or small, addition of 15 mg P L^{-1} or more would be toxic to some plants, and this toxicity would reduce any beneficial Al effects. Phosphorus supply has been associated historically with root growth (Tisdale and Nelson, 1975). In Al phytotoxicity studies, many researchers have used root elongation as a main parameter. Therefore, levels of P supply may have been a very important factor influencing the conclusions of Al phytotoxicity studies. Ideally, it should be best to have the same P concentration for all levels of Al.

Table 3-1. Effects of pH, and additions of Al and P, on the concentrations of Al and P, and on EC, in filtrates (aged for 7 days at 25°C).

pH	Al addition, mg L^{-1}: 0		5		25		50		100		500	
	P addition, mg L^{-1}: 3	15	3	15	3	15	3	15	3	15	3	15
	Al concentration, mg L^{-1}											
3.5	0.1	0.1	4.7	5.0	25.1	22.5	50.2	46.6	93.5	85.3	493.2	487.5
4.0	0.1	0.1	2.9	1.3	17.5	8.6	46.7	26.4	78.0	66.5	385.0	270.9
4.5	0.1	0.1	0.9	0.2	5.1	1.6	7.1	4.0	8.6	8.0	21.0	19.2
4.8	0.1	0.0	0.2	0.0	0.4	0.2	0.7	0.3	1.7	0.9	3.6	2.1
	P concentration, mg L^{-1}											
3.5	3.1	15.1	3.0	14.5	2.9	9.6	2.7	8.5	2.5	8.6	2.3	8.8
4.0	3.1	15.1	2.1	13.0	0.1	1.1	0.3	0.5	0.4	0.5	0.3	0.5
4.5	3.1	15.2	0.6	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4.8	3.0	14.9	0.1	7.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	EC, dS m^{-1}											
3.5	0.80	0.83	0.89	0.91	0.99	1.02	1.06	1.09	1.26	1.30	2.56	2.64
4.0	0.78	0.81	0.81	0.81	0.97	1.02	1.05	1.10	1.30	1.33	3.19	3.28
4.5	0.74	0.82	0.78	0.80	0.96	1.05	1.10	1.14	1.58	1.60	4.10	4.14
4.8	0.78	0.79	0.87	0.94	0.98	1.14	1.16	1.18	1.60	1.66	4.38	4.47

Table 3-2. Equations describing effects of pH(x_1), Al addition (x_2), and P addition (x_3) on concentration of Al (Y_1), concentration of P (Y_2) and EC (Y_3) in filtrates obtained during Experiment 1 (aged for 7 days at 25°C).

Regression equations	R^2
$Y_1 = 0.36 + 4.048x_2 - 0.853x_1x_2 - 0.023\ x_2x_3 + 0.004\ x_1x_2x_3$	0.97†
$Y_2 = 2.06 + 1.9062x_3 - 0.0720x_2 + 0.0001x_2^2 - 0.3367x_1x_3 - 0.0002x_1x_2x_3$	0.63
$Y_3 = 2.80 - 0.956091x_1 - 0.005863x_2 + 0.111680x_1^2 + 0.000228x_3^2 - 0.000002x_2^2 + 0.002962x_1x_2$	0.99

† All values of R^2 are significant at $P < 0.001$.

The EC increased with increasing levels of Al and P addition. Increased pH caused a rapid increase of EC when Al or P additions were large. Also, Blamey et al. (1983) reported that the EC of basal solutions affected the concentrations of Al and P in solution.

After aging for 7 days, the Al and P concentrations and EC in the filtered nutrient solutions and the supernatant solutions were the same. Therefore, in the greenhouse studies with large volumes of culture solution, supernatant liquid in the mixture could be siphoned instead of filtered for convenience. There were no significant differences in Al and P concentrations and EC between 7 and 14 days of aging in the range of pH, Al and P additions of this experiment. The 7-day aging temperatures of 5, 25, and 45°C had no significant effect, either. The Al and P concentrations and EC in the filtered nutrient solution did not change after storage in tightly closed bottles for 20 days.

Experiment 2. Supernatant Solution Containing Several Levels of Al and Similar Concentrations of P

At certain pH levels, and when Al addition was small, the P concentrations in the filtered nutrient solution increased continuously with increased levels of P addition. When large amounts of Al were added, the P concentrations went up and down several times with increased P addition (Fig. 3-1). At a certain P concentration, there might be more than one P addition and more than one corresponding Al concentration which decreased continuously. At different pH values, however, the upper limits of Al addition for continuous increases of P concentrations were different (i.e., the higher the pH, the smaller the upper limit of Al addition). For example, at pH 4.0 and with a 20 mg Al L^{-1} addition, the P concentration still

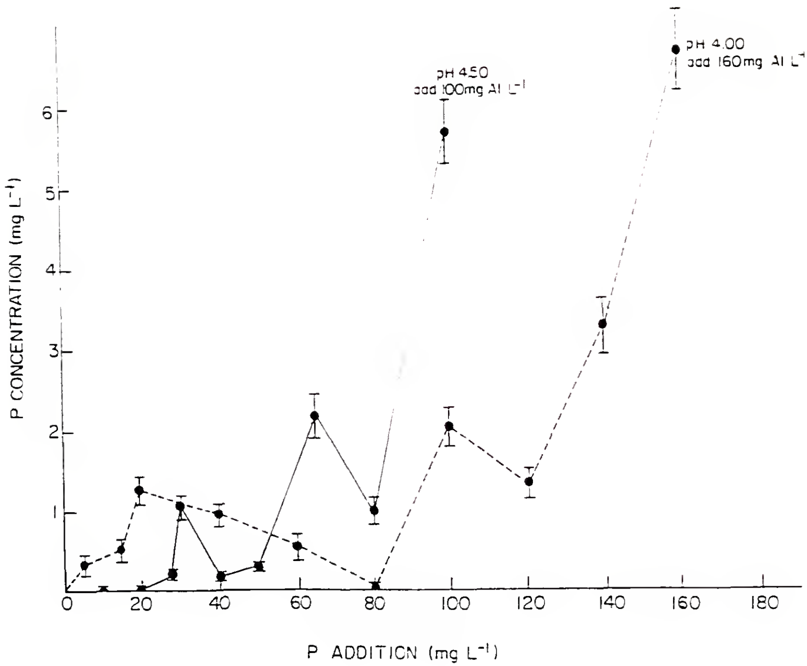

Figure 3-1. Phosphorus concentration in filtered nutrient solution as affected by P addition at two pH values and two levels of Al addition (aged for 7 days at 25°C). Vertical bars indicate standard deviations.

continuously increased with increased P additions. However, at pH 4.5 when Al addition was only 15 mg Al L^{-1}, the P concentration was not increased continuously with increased P additions. An attempt to interpret these results in terms of the prevailing concept of Al^{3+} hydrolysis, and of complexation by Al and P, has not been successful.

According to results from mixing the solutions having a number of levels of Al addition and a number of levels of P addition at two pH values, two sets of filtered nutrient solutions for the Al study were found (Table 3-3). In Set A, the filtered solution contained various levels of Al (0.1 to 171.3 mg Al L^{-1}), but all P concentrations were about 1 mg L^{-1}. The concentrations of other nutrients, and EC of the filtered solution, were adequate for plant growth. This set of solutions may be suitable for large seedlings (e.g., tree seedlings) which are more tolerant to Al and which need more P. In Set B, the pH was 4.5. Aluminum levels ranged from 0.1 to 10.2 mg Al L^{-1} and P concentrations were kept at 0.2 mg P L^{-1} in all treatments. The concentrations of other nutrients and the value of EC were also adequate. This set of supernatant solutions may be suitable for small seedlings which are more sensitive to Al, need less P, and require higher pH. Seedlings of some cereals and vegetables may adapt to this set of solutions.

Experiment 3. Elemental Composition of Large-volume Supernatant Solutions Prepared Manually in the Greenhouse

The nutrients added to solutions and their concentrations in supernatant solutions are shown in Table 3-4. The pH and additions of Al and P were the same as in Set A of Experiment 2. However, Al, P, and Fe concentrations, and EC values, were different from those of

Table 3-3. Elemental additions to solution and concentrations in filtrates at two pH values after aging for 7 days at 25°C (mixed solution with magnetic stirrers).

Set No.	pH	Addition to solution† Al	Addition to solution† P	Concentration in filtrate Al	P	NH_4	NO_3	Ca	Mg	K	Fe	Zn	Cu	Mn	B	EC of filtrate
		mg L^{-1}														dS m^{-1}
A	4.0	0	0.9	0.1	1.0	24	25	50	16	53	0.9	0.1	0.02	0.2	0.2	0.77
		20	16	3.1	1.2	24	25	49	16	53	0.6	0.1	0.02	0.2	0.2	1.00
		80	60	5.1	1.1	24	25	49	16	53	0.5	0.1	0.02	0.2	0.2	1.59
		160	90	14.0	0.8	24	25	49	16	53	0.5	0.1	0.02	0.2	0.2	2.46
		220	85	42.5	0.8	24	25	49	16	53	0.5	0.1	0.02	0.2	0.2	2.74
		270	75	77.1	1.0	24	26	49	16	53	0.5	0.1	0.02	0.2	0.2	2.87
		320	27	171.3	1.2	25	26	49	16	53	0.5	0.1	0.02	0.2	0.2	2.94
B	4.5	0	0.2	0.1	0.2	24	25	50	16	53	0.9	0.1	0.02	0.2	0.2	0.73
		1	0.5	0.8	0.2	24	25	50	16	53	0.9	0.1	0.02	0.2	0.2	0.75
		30	17	1.6	0.2	24	25	49	16	53	0.7	0.1	0.02	0.2	0.2	1.08
		60	32	2.2	0.2	24	25	49	16	53	0.5	0.1	0.02	0.2	0.2	1.29
		100	28	6.0	0.2	24	25	49	16	53	0.4	0.1	0.02	0.2	0.2	1.65
		130	26	7.2	0.2	24	25	48	16	53	0.3	0.1	0.02	0.2	0.2	1.91
		160	25	10.2	0.2	24	25	48	16	53	0.3	0.1	0.02	0.2	0.2	2.17

† Elemental additions to the nutrient solution were as follows (mg L^{-1}): 50 N as NH_4NO_3, 50 K as K_2SO_4, 50 Ca as $CaCl_2$, 15 Mg as $MgSO_4 \cdot 7H_2O$, 2.0 Fe as $FeSO_4 \cdot 7H_2O$, 0.2 Mn as $MnSO_4 \cdot H_2O$, 0.2 B as H_3BO_4, 0.1 Zn as $ZnSO_4 \cdot 7H_2O$, 0.02 Cu as $CuSO_4 \cdot 5H_2O$, and 0.02 Mo as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Al was added as $Al_2(SO_4)_3 \cdot 18H_2O$. P was added as $NaH_2PO_4 \cdot H_2O$.

Table 3-4. Elemental additions to solution and concentrations in supernatants at pH 4.0 after aging for 7 days at room temperature (mixed solution manually).

Treatment	Addition to solution†		Concentration in supernatant												EC of super-natant
	Al	P	Al	P	NH_4	NO_3	Ca	Mg	K	Fe	Zn	Cu	Mn	B	
	mg L^{-1}														dS m^{-1}
Al-0	0	0.9	0.1	0.9	24	25	50	15	53	1.2	0.1	0.02	0.2	0.2	0.77
Al-1	20	16	2.9	1.2	24	25	49	15	53	0.8	0.1	0.02	0.2	0.2	1.00
Al-2	80	60	5.0	1.1	24	25	49	15	53	0.8	0.1	0.02	0.2	0.2	1.65
Al-3	160	90	8.4	0.8	24	25	49	15	53	0.8	0.1	0.02	0.2	0.2	2.38
Al-4	220	85	24.5	0.7	24	25	49	15	53	0.7	0.1	0.02	0.2	0.2	2.65
Al-5	270	75	28.5	0.7	24	26	49	15	53	0.7	0.1	0.02	0.2	0.2	2.75
Al-6	320	27	44.7	0.7	25	26	49	15	53	0.7	0.1	0.02	0.2	0.2	2.82

† Elemental additions to the nutrient solution were as follows (mg L^{-1}): 50 N as NH_4NO_3, 50 K as K_2SO_4, 50 Ca as $CaCl_2$, 15 Mg as $MgSO_4 \cdot 7H_2O$, 2.0 Fe as $FeSO_4 \cdot 7H_2O$, 0.2 Mn as $MnSO_4 \cdot H_2O$, 0.2 B as H_3BO_4, 0.1 Zn as $ZnSO_4 \cdot 7H_2O$, 0.02 Cu as $CuSO_4 \cdot 5H_2O$, and 0.02 Mo as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Al was added as $Al_2(SO_4)_3 \cdot 18H_2O$. P was added as $NaH_2PO_4 \cdot H_2O$.

Set A. Electrical conductivities were slightly lower and Fe concentrations slightly higher than those in Set A. When levels of Al addition were large, P concentrations slightly decreased. The big change from Set A in Experiment 2 was in Al concentrations, which ranged only from 0.1 to 44.8 mg Al L^{-1}. The main causes for the big difference in Al concentrations between the supernatants prepared in Experiments 2 and 3 were the methods used in adjusting the pH of the solution and mixing the solution. In the preparation of the equilibrium solution for Experiment 3, more concentrated NaOH solution was used. Furthermore, a larger amount of NaOH was added each time in Experiment 3 than in Experiment 2, and the added NaOH could not be mixed immediately and thoroughly. The local higher concentration of NaOH reacted with Al^{3+} to form greater amounts of amorphous hydroxy-aluminum and, thus, soluble monomeric Al decreased. The large amount of amorphous hydroxy-aluminum in solutions representing high Al treatments reacted almost immediately with phosphate to form precipitates. In contrast, more Al^{3+} in the Set A solutions of Experiment 2 was reacted with phosphate to form soluble complexes (Hsu, 1968). Therefore, the formation of large amounts of amorphous hydroxy-aluminum caused decreased Al and P concentrations in the supernatant solution. However, the P-concentration decrease was small and all of the P concentrations were about 1 mg L^{-1}. This supernatant solution containing various Al levels and yet similar P concentrations should be suitable for Al phytotoxicity studies.

Experiment 4. Test of Supernatant Solutions as Culture Solutions Using Citrus Seedlings

Citrus seedlings were grown in the supernatant solution prepared in Experiment 3 for 60 days. The seedlings grew rapidly during the

last 20-day period. The data for Rough lemon were chosen to show the shoot height at the beginning and the new-growth shoot height at the end of the third 20-day period (Table 3-5). The 2.7 and 4.8 mg Al L^{-1} treatments appeared to have had a beneficial effect, but 8.3 mg Al L^{-1} or more had a toxic effect on shoot growth. Figure 3-2 shows that the beneficial and toxic effects of different Al concentrations on Rough lemon were obvious. Growth for the other four rootstocks was similar to that for Rough lemon. All seedlings responded significantly to Al concentrations and no symptoms of nutrient deficiencies or excesses were found, except that some seedlings (Swingle citrumelo) showed yellow color, and mottled and withered young leaves and aborted terminals in the high-Al treatments near the end of 60 days. These symptoms may have been caused by Al toxicity.

The pH of the supernatant solution during the growth period normally changed ±0.2 units every 3 days. When the roots grew vigorously at low Al levels near the end of 60 days, the pH decrease of the solution was greater. This was probably due to the acid exudate of the citrus roots. During this period, the pH was adjusted daily.

The nutrient composition of the supernatant solution was analyzed at the end of the last 20-day period, with results as shown in Table 3-6. Aluminum concentration changed very little. The amounts of nutrients remaining in the supernatant solution indicated that the supernatant solution had the capacity to support large seedlings growing for 20 days. For 7-month-old citrus seedlings, it appears that more than 25 mg NH_4^+-N L^{-1} should be applied.

Table 3-5. Shoot height and new-growth shoot height of Rough lemon in the 3rd 20-day growth period in the supernatant solution.

Treatment	Al concentration in supernatant solution†	Shoot height at the beginning	New-growth shoot height at the end
	mg L^{-1}	------------cm $plant^{-1}$	---------------
Al-0	0.1	26.6±2.7‡	10.7±1.1
Al-1	2.7	29.6±3.0	13.7±1.6
Al-2	4.8	28.4±2.6	12.5±1.4
Al-3	8.3	21.7±1.3	5.8±0.5
Al-4	24.4	16.8±0.8	0.9±0.1
Al-5	28.4	16.7±0.7	0.8±0.1
Al-6	44.6	16.7±0.7	0.8±0.1

† Aluminum concentration taken as the average of Al concentrations at the beginning and at the end of the 3rd 20-day growth period.

‡ ± Standard deviation.

Figure 3-2. Eight-month-old Rough lemon seedlings grown for 60 days in supernatant solution with various concentrations of Al. From left to right: 0.1, 2.7, 4.8, 8.3, 24.4, 28.4, and 44.6 mg Al L^{-1}.

Table 3-6. Elemental concentrations and EC of supernatant solution after growing five 7-month-old citrus seedlings in ten liters of supernatant solution for 20 days.

Treat-ment	Al	P	NH_4	NO_3	Ca	Mg	K	Fe	Zn	Cu	Mn	B	EC
	mg L^{-1}												dS m^{-1}
Al-0	0.1	0.7	9	18	48	14	42	0.6	0.1	0.01	0.1	0.1	0.64
Al-1	2.5	1.0	6	14	45	14	38	0.3	0.2	0.01	0.2	0.1	0.82
Al-2	4.6	0.9	7	16	46	14	39	0.4	0.2	0.01	0.2	0.1	1.47
Al-3	8.2	0.7	11	18	47	14	46	0.5	0.2	0.01	0.2	0.1	2.25
Al-4	24.0	0.7	14	19	48	14	48	0.5	0.2	0.02	0.2	0.2	2.47
Al-5	28.2	0.7	16	21	48	14	49	0.5	0.2	0.02	0.2	0.2	2.60
Al-6	44.4	0.7	18	22	48	14	50	0.6	0.2	0.02	0.2	0.2	2.64

Summary and Conclusions

Precipitation of $Al(OH)_3$ and aluminum phosphate may occur in nutrient solution if a large amount of Al and P have been added at a relatively high pH. The objective of this study was to investigate the actual concentrations of Al and P in nutrient solution under different pH conditions and varied levels of Al and P addition, and to develop and test a supernatant-solution method for Al studies in which the supernatant solutions contained various levels of Al and similar concentrations of P. The aluminum concentration in supernatant solutions was greatly reduced when pH was adjusted to 4.5 or higher. Phosphorus concentration became negligible when pH was 4.5 or higher and Al addition was 25 mg L^{-1} or more, even when 15 mg P L^{-1} was added to the solution. The large changes in P concentration of the supernatant solution may confound the apparent effects of Al on plant growth. Two sets of supernatant solutions which contained various levels of Al and similar concentrations of P at two pH levels were developed. One set of the supernatant solutions with pH 4.0 was used in the greenhouse study to test suitability of the supernatant solutions as culture solutions for Al phytotoxicity studies. Results showed that the supernatant-solution technique was successful.

Two sets of supernatant solutions are recommended for Al phytotoxicity studies. In the pH 4.0 set, Al additions as $Al_2(SO_4)_3 \cdot 18H_2O$ are 0, 20, 80, 160, 220, 270, and 320 mg Al L^{-1} and corresponding P additions as $NaH_2PO_4 \cdot H_2O$ are 0.9, 16, 60, 90, 85, 75, and 27 mg P L^{-1}. The maximum Al concentration will be 171 mg Al L^{-1} and the P concentration will be about 1 mg P L^{-1} in all treatments. This set

is suitable for larger seedlings. In the pH 4.5 set, Al additions are 0, 1, 30, 60, 100, 130, and 160 mg Al L^{-1} and corresponding P additions are 0.25, 0.50, 12, 32, 28, 26, and 25 mg P L^{-1}. The maximum Al concentration will be 10 mg Al L^{-1} and the P concentration will be about 0.2 mg P L^{-1} in all treatments. The concentrations of Al and P are affected by the preparation procedure, such as the concentrations of alkali and acid used to adjust pH and the speed of mixing for this solution. This supernatant-solution method makes it possible to avoid the confounding effects of P on Al, and to report the actual concentration of Al in solution. Also, this method and the use of regression procedures make it possible to obtain critical values of Al concentration of toxic effects to plant growth.

CHAPTER IV

DIFFERENTIAL RESPONSE OF CITRUS ROOTSTOCKS TO ALUMINUM LEVELS IN NUTRIENT SOLUTIONS

Introduction

Few researchers have studied the effects of Al on citrus rootstocks in nutrient solutions. Haas (1936) used leafy-twig cuttings of lemon, Lisbon, and Valencia orange in a nutrient solution and found that, when Al was present the citrus roots were healthy, more extensive, and root caps were numerous, but the shoots usually were retarded. He concluded that "a concentration of 15 to 20 mg L^{-1} of Al was rather high for the production of the greatest growth" (tops and roots). His data showed that the addition of Al to the solution increased the percentage of P in root dry matter. Liebig et al. (1942) found that the addition of 2.5 to 5 mg Al L^{-1} to nutrient solutions greatly stimulated root development but depressed shoot growth of Valencia orange and lemon cuttings. Lower concentrations of Al, i.e., 0.1 and 0.5 mg L^{-1}, did not produce this effect. They found an antagonistic effect of Al on Cu. Yokomizo and Ishihara (1973) concluded that root growth of Natsudaidai (*C. Natsudaida* Hayata) seedlings improved at low concentration of Al but began to decrease at an Al addition of 20 mg L^{-1} and was extremely depressed at 100 mg L^{-1} in nutrient solution. Root growth of Satsuma mandarin trees was apparently increased by the supply of 10 and 20 mg Al L^{-1}. However, no results have been reported in the literature for

systematic screening of citrus rootstocks for Al tolerance. The Al phytotoxicity levels are still not known for many citrus rootstocks, and few data exist on effects of Al on mineral nutrition of citrus.

A general problem with previous work was that the authors only reported the amounts of Al added to the solution, not the actual concentrations in the nutrient solution. The actual concentrations of Al in the nutrient solution were always lower than the original concentration due to precipitation of $Al(OH)_3$ and aluminum phosphate (Yokomizo and Ishihara, 1973; Blamey et al., 1983; Bollard, 1983). Another problem is the confounding effects of P on Al effects on plant growth. The use of a supernatant-nutrient-solution method developed in Chapter III might help to resolve these problems.

The objectives of this study were (1) to evaluate root and shoot growth responses of citrus rootstocks to different Al concentrations in nutrient solution; (2) to estimate the critical Al phytotoxicity concentrations for citrus rootstocks grown in Florida; and (3) to evaluate the relationships between elemental concentrations in plant tissue and Al effects on plant growth.

Materials and Methods

Plant Material

Five citrus rootstocks were evaluated which account for more than 90% of the citrus rootstocks used in Florida (Fisher, 1988). They included: (1) Carrizo citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.], (2) Cleopatra mandarin (*C. areshni* Hort. ex Tanaka), (3) Rough lemon (*C. jambhiri* Lush.), (4) Sour orange (*C. aurantium* L.), and (5) Swingle citrumelo (*C. paradisi* x

P. trifoliata). Six-month-old seedlings were obtained from nurseries. Uniform seedlings were selected, washed thoroughly with tap water, and rinsed with deionized water. The root system of each seedling was spread on a 1-cm grid background and photographed. The photographs were enlarged and used to measure the original root length (Tennant, 1975). The fresh weight of whole plant and shoot height of seedlings were recorded. The seedlings were then transferred to nutrient solutions.

Nutrient Solution

The first set of supernatant nutrient solutions recommended in Chapter III was used. The procedure of nutrient-solution preparation was the same as described in Experiment 3, Chapter III. The elemental additions and concentrations in the supernatant solutions were as shown in Table 3-4. The seven Al concentrations in the supernatant solutions ranged from 0.1 to 44.8 mg Al L^{-1}, with P concentrations of about 1 mg P L^{-1} in all treatments. The nutrient solution was replaced each 20 days (three total replacements). Before the plants were put into the solutions, and after each 20-day growth period, aliquots of the solutions were taken for Al analysis. The initial and final Al concentrations were averaged and were assumed to represent the Al concentration during this 20-day growth period. Three 20-day Al concentrations were averaged and the mean was taken as the Al level during the 60-day growth period.

Equipment

Ten-liter pails were used to hold the nutrient solution. Five holes were made in the plywood lids of the pails, one for each seedling. A seedling was held in the hole by a rubber stopper cut

into two parts, with a small hole in the middle. The entire root system of a seedling was submerged in the solution. One small hole was also made in the lid for inserting an aeration tube. Continuous aeration was supplied by an air pump hooked up to plastic tubes which were attached to airstones in the solution. This also served to keep the solution uniformly mixed (Fig. 4-1).

The toxicity of a given concentration of Al is highly temperature-dependent (Konzak et al., 1976; Aniol, 1983). In the present study, air-conditioning was used in the greenhouse to maintain the air temperature between 25 and 38°C. Plastic-lined pools were also set up on the benches in the greenhouse, with the pails then put inside the pools filled with water. The water in the pools was circulated by a pump and passed through a cooling system. The temperature of the water in the pools and in the nutrient solution in the pails was maintained at 25±1°C (Fig. 4-2).

Procedure

The study was conducted using a split-plot design with 7 Al levels as the whole plot, completely randomized in 8 replications with 5 rootstocks as the subplot. Eight pails contained the same concentration of Al and 5 rootstock seedlings. A total of 56 pails containing treatment nutrient solutions were randomly assigned to positions in the pools (Fig. 4-3).

The pH levels were checked every 2 or 3 days and adjusted to 4.0 by HCl or NaOH additions when necessary. The solution level was maintained by addition of deionized water in quantity sufficient to offset loss due to transpiration every two days. The seedlings grew in the solution from June 2 to August 2, 1989.

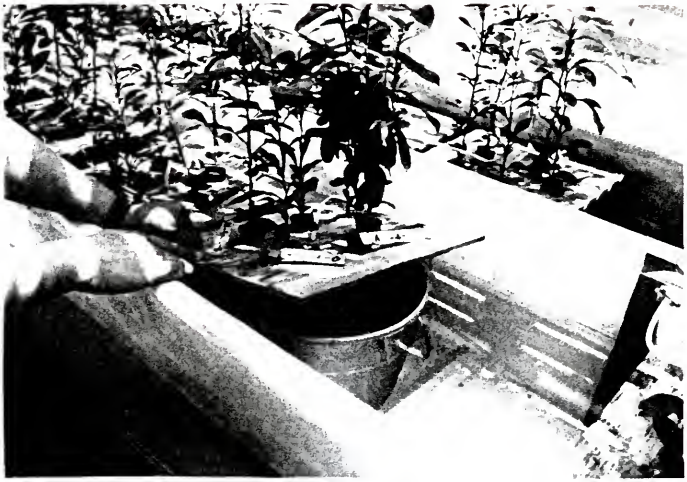

Figure 4-1. The root systems of five citrus seedlings which were submerged in nutrient solutions in pails which were in turn placed in a water pool.

Figure 4-2. Water circulation in the pool by a pump, which was maintained at 25±1°C by passing through a cooling system.

Figure 4-3. Citrus seedlings growing in nutrient solutions in pails which were randomly assigned to positions in the water-filled pool.

At the end of the experiment, plants were washed thoroughly with tap water and given a final rinsing with deionized water. The root and shoot morphology was assessed visually and from photographs. Total root length, shoot height, and fresh weight of whole seedling were measured. The differences between initial and final measurements were considered as new-growth root length, new-growth shoot height, and new-growth fresh weight. Roots and shoots were dried and analyzed for elemental concentrations. Because of the small quantities of some seedlings, the roots or shoots of the eight replications of a given treatment were randomly combined into four samples, respectively. The roots or shoots were ground to pass a 0.85-mm sieve. Tissue samples of 0.500 g were dry-ashed at 500°C in a muffle furnace for 4 h; the ash was then dissolved in 10 mL of 6 M HCl, evaporated to dryness, and the temperature increased slightly to dehydrate SiO_2. The residue was dissolved in 6.7 mL of 2 M HCl, heated to near-boiling, and then filtered. Elemental concentrations in the solution were determined by inductively coupled argon plasma (ICAP) emission spectroscopy.

Regression/correlation techniques were employed to relate growth to Al concentrations in nutrient solution. The growth data were transformed to natural logs and the Al concentrations to square-root values.

Results and Discussion

Morphology of Roots and Shoots as Affected by Al Concentration

At 2.7 and 4.8 mg Al L^{-1}, the roots of all rootstocks except Carrizo citrange grew extremely well. The roots appeared whiter, healthier, firmer, and straighter than those in 0.1 mg Al L^{-1}.

More new roots and lateral roots grew and, near the end of the experiment, the roots of Rough lemon grew fastest among the five rootstocks. When the Al concentration was 8.3 mg L^{-1} or higher, the growth of roots was retarded. Fewer new roots and lateral roots grew and root tips became thickened (Fig. 4-4). At the 28.4 and 44.6 mg Al L^{-1} levels, the root system as a whole appeared coralloid, with stubby new-growth roots (Fig. 4-5). At the 44.6 mg Al L^{-1} level, some older roots rotted, with Rough lemon roots deteriorating most seriously. At 8.3 mg Al L^{-1} or higher, some root tips were covered by a root cap with black gelatinous material (Fig. 4-6). The number of blackened root caps increased with increased Al concentration in solution. Among the five rootstocks, Cleopatra mandarin had the greatest number of blackened root caps. It appeared that the rootstock which was more tolerant to Al had more of this kind of root cap. Therefore, the black gelatinous material on the root cap might be related to avoidance of Al toxicity. What the black gelatinous material was, how it formed, and what its function was, however, were not known. The black gelatinous material was probably the excreta of roots or complexes of the excreta with some components in the solution, such as Al.

Shoots of the seedlings grew faster in the 2.7 mg Al L^{-1} treatment than in the 0.1 mg Al L^{-1} treatment. When Al concentration was 8.3 mg L^{-1} or higher, growth of shoots was retarded. Shoots of five rootstock seedlings were shorter and leaves were fewer and smaller (Fig. 4-7) on plants grown of the higher Al concentrations. However, no Al toxicity symptoms or other elemental toxicity or deficient symptoms were observed during the first 50 days. Near the end of the

Figure 4-4. Thickened root tips of Sour orange seedlings grown in solution with 24.4 mg Al L^{-1}.

Figure 4-5. Stubby new-growth roots of Rough lemon seedlings grown in nutrient solution with 24.4 mg Al L^{-1}.

Figure 4-6. Root tip covered by a root cap with black gelatinous material for Cleopatra mandarin seedlings grown in solution at 24.4 mg Al L^{-1}.

Figure 4-7. Young leaves of Swingle citrumelo seedlings grown in nutrient solutions with various concentrations of Al. From left to right: 0.1, 2.7, 24.4, and 28.4 mg Al L^{-1}.

Figure 4-8. Shoot with yellow, mottled, and withered young leaves and aborted terminal of Swingle citrumelo seedling grown in nutrient solution with 44.6 mg Al L^{-1} for 60 days.

experiment, at 24.4 mg Al L^{-1} or higher, young leaves of Swingle citrumelo were yellow, mottled and withered. Furthermore, the terminal shoot was aborted (Fig. 4-8). Rough lemon had similar symptoms but the symptoms were much less pronounced. These symptoms were different from the symptoms of elemental deficiency or excess for citrus, as listed by Chapman (1968). The symptoms were probably caused by Al toxicity.

Growth Responses of Citrus Seedlings to Al Concentrations in Nutrient Solution

Growth of five rootstock seedlings for 60 days in the nutrient solution with various concentrations of Al is shown in Figs. 4-9 through 4-13. It was obvious that the growth of roots and shoots was different among the Al treatments. The differences between the 2.7 mg Al L^{-1} and 44.6 mg Al L^{-1} treatments were particularly evident.

The initial, final, and new-growth of three parameters for five rootstock seedlings were listed in Appendix (Table A-1). The effects of Al concentration on new-growth root length of five rootstock seedlings are shown in Fig. 4-14 and the linear regression equations are given in Table 1. Aluminum concentration in the first treatment was so low (0.1 mg Al L^{-1}, i.e., $(Al)^{1/2} = 0.32$) that this concentration would not produce any beneficial or toxic effect on citrus root growth (Liebig et al., 1942). Therefore, the new-growth root length of this treatment was taken as a control. The Al concentration at which the new-growth root length was equal to that of a control was considered as the critical Al concentration. Concentrations below or above the critical Al levels would cause beneficial or toxic effects, respectively. In order to get the critical values, regression equations were calculated. For all

Figure 4-9. Effects of increasing Al concentrations in the nutrient solution on root and shoot growth of 8-month-old Carrizo citrange seedlings. From left to right: 0.1, 2.7, 4.8, 8.3, 24.4, 28.4, and 44.6 mg Al L^{-1}.

Figure 4-10. Effects of increasing Al concentrations in the nutrient solution on root and shoot growth of 8-month-old Cleopatra mandarin seedlings. From left to right: 0.1, 2.7, 4.8, 8.3, 24.4, 28.4, and 44.6 mg Al L^{-1}.

Figure 4-11. Effects of increasing Al concentrations in the nutrient solution on root and shoot growth of 8-month-old Sour orange seedlings. From left to right: 0.1, 2.7, 4.8, 8.3, 24.4, 28.4, and 44.6 mg Al L^{-1}.

Figure 4-12. Effects of increasing Al concentrations in the nutrient solution on root and shoot growth of 8-month-old Rough lemon seedlings. From left to right: 0.1, 2.7, 4.8, 8.3, 24.4, 28.4, and 44.6 mg Al L^{-1}.

Figure 4-13. Effects of increasing Al concentrations in the nutrient solution on root and shoot growth of 8-month-old Swingle citrumelo seedlings. From left to right: 0.1, 2.7, 4.8, 8.3, 24.4, 28.4, and 44.6 mg Al L^{-1}.

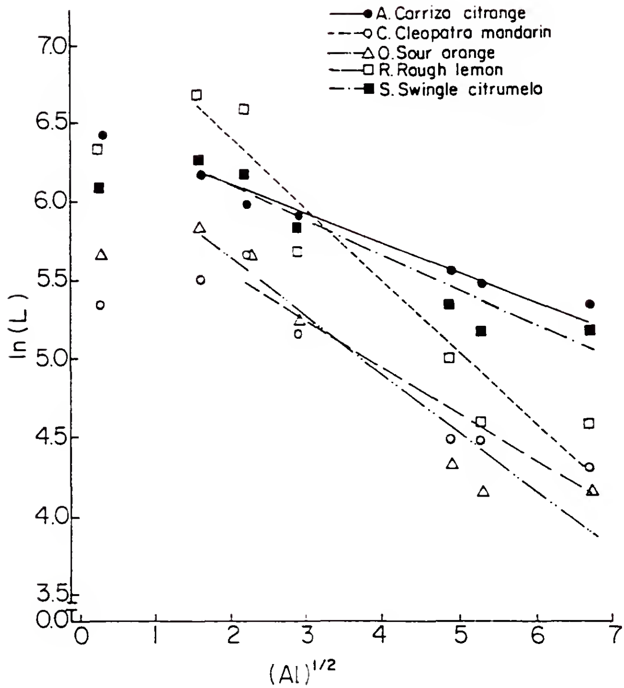

Figure 4-14. Effects of Al concentrations (Al, mg L^{-1}) in nutrient solution on new-growth root length (L, cm $plant^{-1}$) of 8-month-old citrus seedlings grown for 60 days.

Table 4-1. Linear regression equations for prediction of new-growth root length (L, cm plant^{-1}), new-growth shoot height (H, cm plant^{-1}), and new-growth fresh weight (W, g plant^{-1}) of citrus seedlings from Al concentration (Al, mg L^{-1}) in nutrient solution. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo).

Regression equations	r^2	Critical Al concentration
$\ln(L_A) = 6.44 - 0.17(Al)^{1/2}$	0.86†	<2.7
$\ln(L_C) = 6.08 - 0.28(Al)^{1/2}$	0.81	7.2
$\ln(L_O) = 6.40 - 0.38(Al)^{1/2}$	0.89	3.9
$\ln(L_R) = 7.29 - 0.45(Al)^{1/2}$	0.90	4.6
$\ln(L_S) = 6.57 - 0.23(Al)^{1/2}$	0.86	4.5
$\ln(H_A) = 3.93 - 0.60(Al)^{1/2}$	0.92	4.0
$\ln(H_C) = 3.34 - 0.40(Al)^{1/2}$	0.87	8.8
$\ln(H_O) = 3.46 - 0.47(Al)^{1/2}$	0.93	6.0
$\ln(H_R) = 3.93 - 0.60(Al)^{1/2}$	0.81	3.6
$\ln(H_S) = 3.18 - 0.45(Al)^{1/2}$	0.81	1.8
$\ln(W_A) = 2.57 - 0.24(Al)^{1/2}$	0.91	1.8
$\ln(W_C) = 2.20 - 0.24(Al)^{1/2}$	0.79	12.2
$\ln(W_O) = 2.61 - 0.27(Al)^{1/2}$	0.89	5.1
$\ln(W_R) = 3.34 - 0.35(Al)^{1/2}$	0.85	5.1
$\ln(W_S) = 2.53 - 0.17(Al)^{1/2}$	0.72	4.5

†All the values of r^2 were significant at $P < 0.001$.

rootstocks except Carrizo citrange, new-growth root length increased between the control and the treatment which had highest amount of new-growth root length, but the curve was uncertain because there was no treatment between them in most cases. There were 3 or 4 Al treatments between the treatment which had the largest new-growth root length and the treatment with the highest Al concentration in solution, however, and the new-growth root length gradually decreased between these two treatments. Therefore, the regression equation was developed for these 5 or 6 Al treatments. Carrizo citrange was an exception, because new-growth root length gradually decreased from the control to the treatment with the highest Al concentration in solution. According to the trend of the other four rootstocks, it was possible that there might be some Al concentrations lower than the second treatment (2.7 mg Al L^{-1}) which might still have had a beneficial effect on root growth. Therefore, the regression equation was calculated for five treatments from the second treatment (2.7 mg Al L^{-1}) to the last treatment (44.6 mg Al L^{-1}). The same procedure was followed for the other parameters shown in Figs. 4-15 to 4-19, and in Tables 4-1 and 4-2.

The critical concentrations obtained from the regression equations are shown in Table 4-1. The higher critical Al concentrations indicated greater Al tolerance. According to these critical values, the Al tolerance for root growth was as follows (from most tolerant to least tolerant): Cleopatra mandarin > Rough lemon > Swingle citrumelo > Sour orange > Carrizo citrange. The effects of Al concentrations on relative new-growth root length are shown in Fig. 4-15. The root length for the first treatment (0.1 mg Al L^{-1}) was

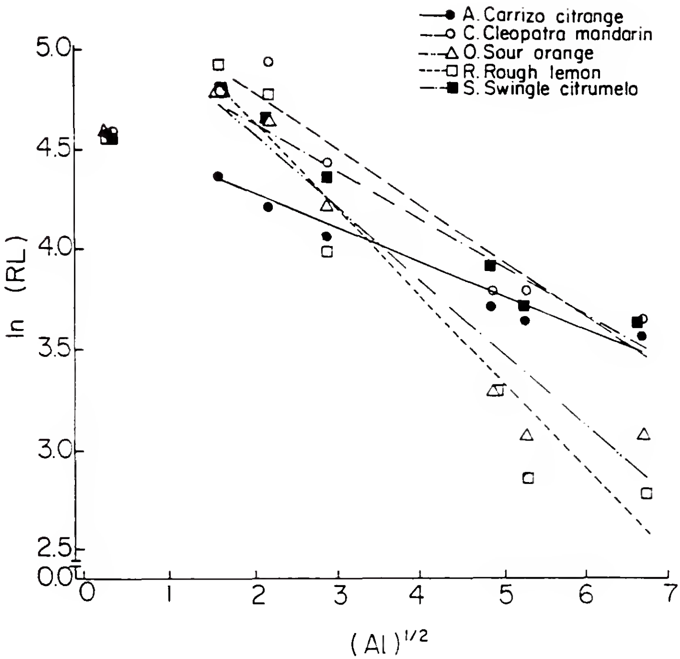

Figure 4-15. Effects of Al concentrations (Al, mg L^{-1}) in nutrient solution on relative new-growth root length (RL, %) of 8-month-old citrus seedlings grown for 60 days.

taken as 100%. According to the predicted highest percentage values shown in Table 4-2, the beneficial effects of low Al concentrations on root growth of five rootstocks were as follows (from most to least beneficial): Rough lemon > Cleopatra mandarin > Sour orange > Swingle citrumelo > Carrizo citrange. This order was somewhat different from the tolerance order. Such difference indicated that the degree of beneficial effect on root growth at low Al concentrations did not correspond well with the sequential order of tolerance. According to the predicted lowest percentage values shown in Table 4-2, the toxic effects of high Al concentrations on root growth of five rootstocks were as follows (from most to least toxic): Rough lemon > Sour orange > Cleopatra mandarin > Carrizo citrange > Swingle citrumelo. This order was different from that of the tolerance order or that of beneficial effect. This difference indicated that the degree of toxic effect on root growth at high Al concentrations did not correspond with the tolerance or the degree of beneficial effects. Rough lemon was in the second position in the tolerance order, for example, and had the highest beneficial effect from low Al concentrations, but suffered most from high Al concentrations among the five rootstocks.

The effects of Al concentrations on new-growth shoot height of five rootstock seedlings are shown in Figs. 4-16 to 4-17, with the regression equations being given in Tables 4-1 and 4-2. The effect of Al concentrations on shoot growth showed a different trend from root growth. The orders of tolerance, beneficial effects, and toxic effects for shoot height also were different from those for root growth. Carrizo citrange and Swingle citrumelo are good examples for

Table 4-2. Linear regression equations for prediction of relative new-growth root length (RL, %), relative new-growth shoot height (RH, %), and relative new-growth shoot weight (RW, %) of citrus seedlings from Al concentration (Al, mg L^{-1}) in nutrient solution. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo.)

Regression equations	r^2	Predicted highest percentage‡	Predicted lowest percentage at 44.6 mg Al L^{-1} treatment
$\ln(RL_A) = 4.62 - 0.17(Al)^{1/2}$	0.86†	76.8	32.6
$\ln(RL_C) = 5.35 - 0.28(Al)^{1/2}$	0.81	114.0	32.5
$\ln(RL_O) = 5.35 - 0.38(Al)^{1/2}$	0.89	112.8	16.6
$\ln(RL_R) = 5.56 - 0.45(Al)^{1/2}$	0.90	124.0	12.9
$\ln(RL_S) = 5.09 - 0.23(Al)^{1/2}$	0.86	111.3	35.0
$\ln(RH_A) = 5.81 - 0.60(Al)^{1/2}$	0.92	124.5	6.1
$\ln(RH_C) = 5.80 - 0.40(Al)^{1/2}$	0.87	171.2	22.8
$\ln(RH_O) = 5.75 - 0.47(Al)^{1/2}$	0.93	145.1	13.6
$\ln(RH_R) = 5.73 - 0.60(Al)^{1/2}$	0.81	114.9	5.6
$\ln(RH_S) = 5.20 - 0.45(Al)^{1/2}$	0.81	86.5	9.0
$\ln(RW_A) = 4.92 - 0.24(Al)^{1/2}$	0.89	92.4	27.6
$\ln(RW_C) = 5.42 - 0.24(Al)^{1/2}$	0.79	135.5	45.5
$\ln(RW_O) = 5.21 - 0.27(Al)^{1/2}$	0.89	117.5	30.2
$\ln(RW_R) = 5.38 - 0.35(Al)^{1/2}$	0.85	122.1	21.0
$\ln(RW_S) = 4.95 - 0.17(Al)^{1/2}$	0.72	106.8	45.4

†All the values of r^2 were significant at $P < 0.001$.

‡Predicted highest percentage in the treatment (2.7 or 4.8 mg Al L^{-1}) for which the sample percentage was highest.

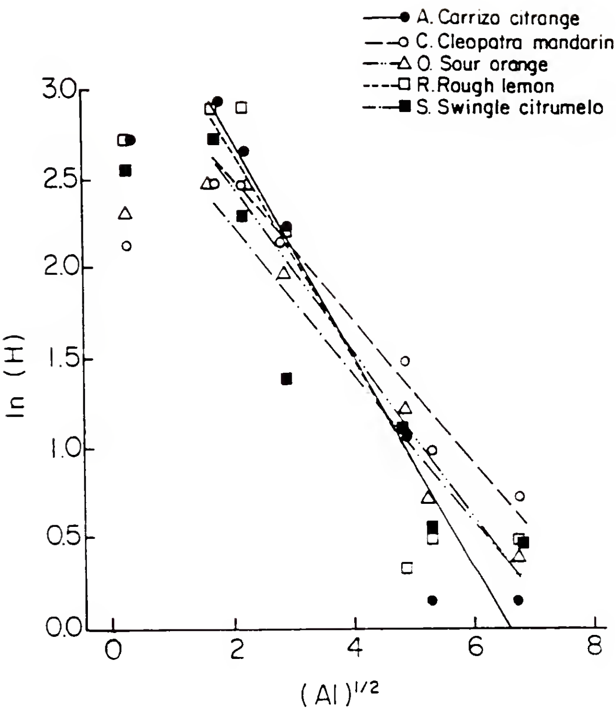

Figure 4-16. Effects of Al concentrations (Al, mg L^{-1}) in nutrient solution on new-growth shoot height (H, cm $plant^{-1}$) of 8-month-old citrus seedlings grown for 60 days.

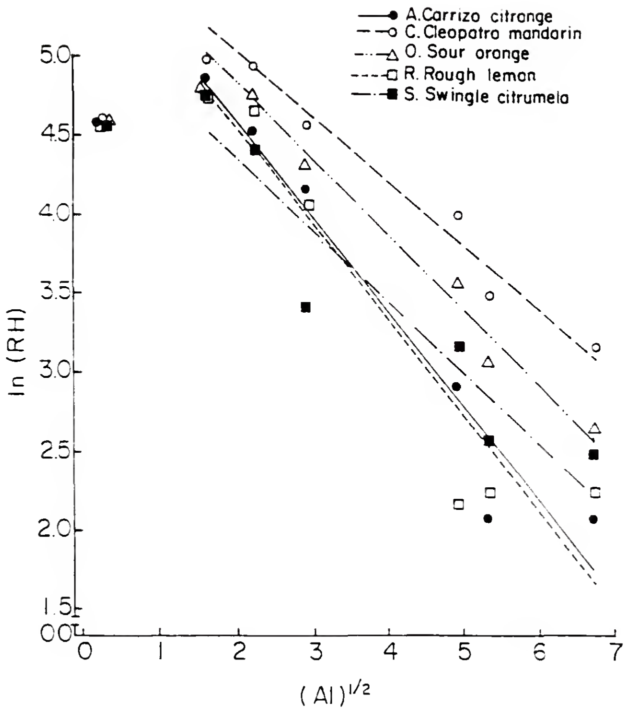

Figure 4-17. Effects of Al concentrations (Al, mg L^{-1}) in nutrient solution on relative new-growth shoot height (RH, %) of 8-month-old citrus seedlings grown for 60 days.

showing such differences. For root growth, Carrizo citrange was least tolerant and did not show a beneficial effect from the 2.7 mg Al L^{-1} treatment. For shoot growth, however, Carrizo citrange had the third position in the tolerance list and showed a beneficial effect at 2.7 mg Al L^{-1}. Swingle citrumelo had the third position in tolerance list and showed a beneficial effect at 2.7 mg Al L^{-1} treatment for root growth. For shoot growth, however, Swingle citrumelo was least tolerant and the predicted relative new-growth shoot height was less than 100%. Haas (1936) and Liebig et al. (1942) also found that low concentrations stimulated root growth but depressed top growth for some citrus species. Several questions need answers: Are those roots developed by stimulation of low Al concentrations normal in their absorption of nutrients from the matrix? Are there any stimulation effects of low Al concentrations on the function of nutrient absorption by roots instead of on the development of root length? Are there different physiological effects of absorbed Al on shoot growth of different citrus rootstocks?

The different growth responses of roots and shoots indicated that neither of these alone was a good indicator for evaluation of the Al tolerance of citrus rootstocks. Because fresh-weight values were the sum for roots and shoots, the fresh-weight response combined the responses of root length and shoot height. In general, new-growth fresh weight of whole plants should be a better indicator for evaluation of Al tolerance than the other two parameters. The effect of Al concentrations on new-growth fresh weight and relative new-growth fresh weight of five rootstock seedlings are shown in Figs. 4-18 and 4-19, with the regression equations being given in Tables 4-1

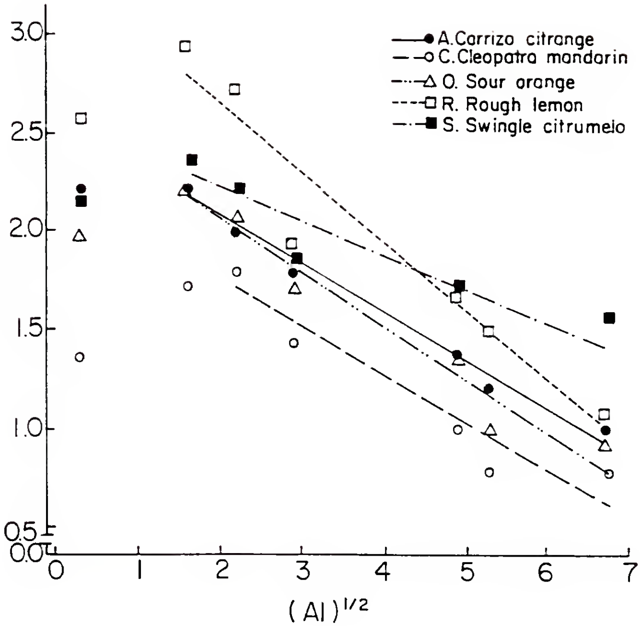

Figure 4-18. Effects of Al concentrations (Al, mg L^{-1}) in nutrient solution on new-growth fresh weight (W, g $plant^{-1}$) of 8-month-old citrus seedlings grown for 60 days.

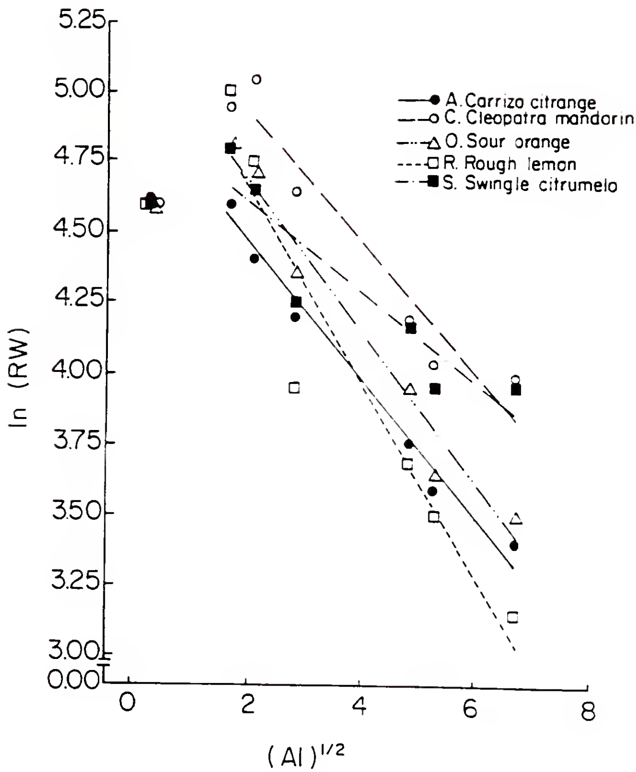

Figure 4-19. Effects of Al concentrations (Al, mg L^{-1}) in nutrient solution on relative new-growth fresh weight (RW, %) of 8-month-old citrus seedlings grown for 60 days.

and 4-2. According to the critical Al concentration, the Al tolerance for fresh weight increase was as follows (from most tolerant to least tolerant): Cleopatra mandarin > Rough lemon = Sour orange > Swingle citrumelo > Carrizo citrange. The benefical-effect order was (from most to least benefical): Cleopatra mandarin > Rough lemon > Sour orange > Swingle citrumelo > Carrizo citrange. This order was similar to the tolerance order. The toxic-effect order was (from most to least toxic): Rough lemon > Carrizo citrange > Sour orange > Swingle citrumelo > Cleopatra mandarin. This order was different from both the tolerance order and the beneficial-effect order.

Elemental Concentrations in Roots and Shoots as Affected by Al Concentrations in Growth Solution.

The elemental concentrations and their standard deviations for roots and shoots of five rootstocks grown in various Al concentrations in solution are listed in the Appendix (Tables A-2a and A-2b). The standard deviations were small, and the coefficients of variation were normally less than 5% for each Al treatment and each element (4 replications). The elemental concentrations in roots and shoots as affected by Al concentrations in the growth solution are shown in Figs. 4-20 to 4-28.

Aluminum

At the 0.1 mg Al L^{-1} level, the Al concentrations of roots of all five rootstocks were similar (about 45 mg Al kg^{-1}). When Al concentrations in solution increased, furthermore, the Al concentrations of roots of five rootstocks were increased (Fig. 4-20). When Al concentrations were 2.7, 4.8, and 8.3 mg Al L^{-1} in solution, Rough lemon and Cleopatra mandarin had higher Al concentrations than the others, while Carrizo citrange had the lowest concentration among the

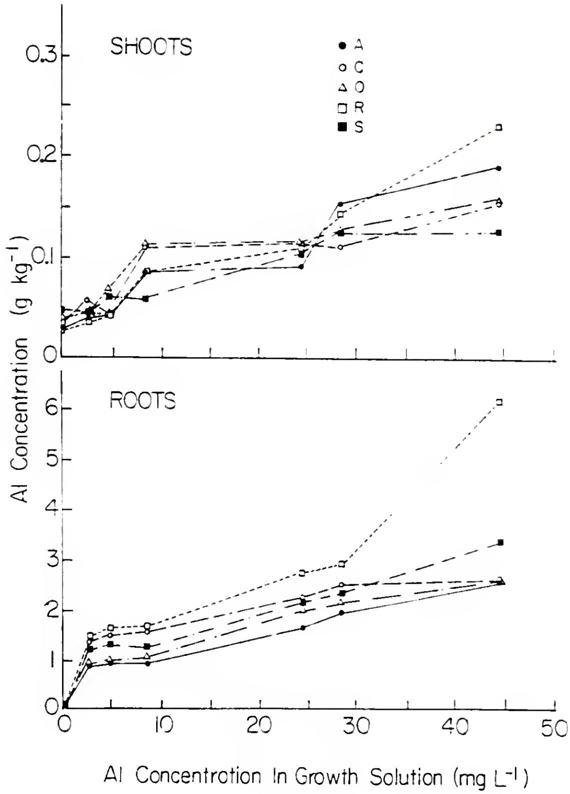

Figure 4-20. Aluminum concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

five rootstocks. This trend was similar to those for tolerance and for beneficial effects (Table 4-1 and 4-2). It might be concluded that the root Al concentrations were higher for Al-tolerant than for Al-sensitive rootstocks when Al concentrations in solution were 2.7 mg L^{-1} or higher. It might be also concluded that those roots which accumulated more Al had greater new-root growth. These relationships imply that Al accumulation in roots was a characteristic associated with Al beneficial effects and tolerance of roots. When the Al concentrations in roots were high, such as 6165 mg Al kg^{-1} for Rough lemon at the 44.6 mg Al L^{-1} level, the accumulation of Al in roots apparently damaged the roots. The Al concentrations in shoots also increased with increased Al concentration in solution. However, the increases in Al concentration of the shoots were much less than those for the roots. Comparison of Fig. 4-20 and Fig. 4-17, led to the conclusion that there was no certain relation between Al concentration in shoots and Al-beneficial effects and Al-tolerance of rootstocks. The relation between Al concentrations in roots and shoots and Al-tolerance of citrus seedlings did not belong to any of the three groups described by Foy (1984).

Calcium

The Ca concentrations in the roots of the five rootstocks decreased with increased Al concentration in solution up to 8.3 mg L^{-1} (Fig. 4-21). However, when Al concentrations in solution were higher than 8.3 mg L^{-1}, the Ca concentrations in the roots of all rootstocks underwent little further change; i.e., they all remained similar, with the shoots having higher Ca concentrations than the roots.

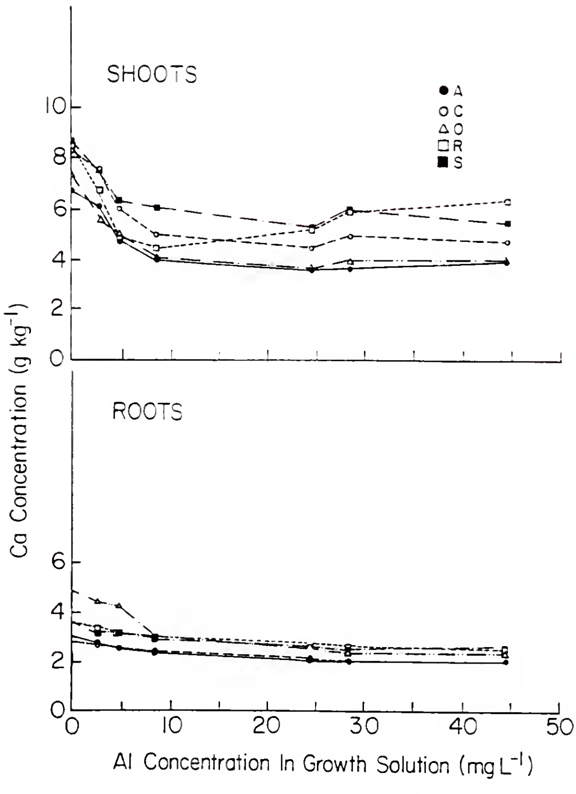

Figure 4-21. Calcium concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

The Al concentrations in the shoots showed the same trend as those in the roots. Al-induced Ca deficiency has been associated with Al toxicity effects (Lance and Pearson, 1969; Lund, 1970). In the present study, the Ca concentrations in roots decreased when Al had a beneficial effect, while such concentrations remained the same when Al had a toxic effect. Therefore, Ca concentrations in citrus plants might not be the main factor related to toxic-Al effects. This conclusion could be applied to Zn, Mn, Cu, and Fe as well in the following discussion. There was no certain relationship between Ca concentrations in roots or shoots and Al-tolerance of citrus rootstocks.

Magnesium

The Mg concentrations in roots of five rootstock seedlings increased when Al concentrations in solution increased to 2.7, 4.8, or 8.3 mg L^{-1}, and then decreased and remained the same when the Al concentrations were higher than 28.4 mg L^{-1} in solution. In contrast, the Mg concentrations in shoots decreased when Al concentration in solution increased up to 8.3 mg L^{-1}. Above that Al concentration, the shoots maintained their Mg concentrations. Swingle citrumelo accumulated more Mg both in roots and shoots than did the others. There was no certain relation between Mg concentrations in roots or shoots and Al-beneficial effects or Al-tolerance of citrus rootstocks.

Potassium and phosphorus

The K and P concentrations in both roots and shoots increased when Al concentrations in solution increased up to 2.7 or 4.8 mg L^{-1} (Figs. 4-23 and 4-24), with the P concentrations increasing rapidly. When the Al concentrations were higher than 4.8 mg L^{-1}, the K and P

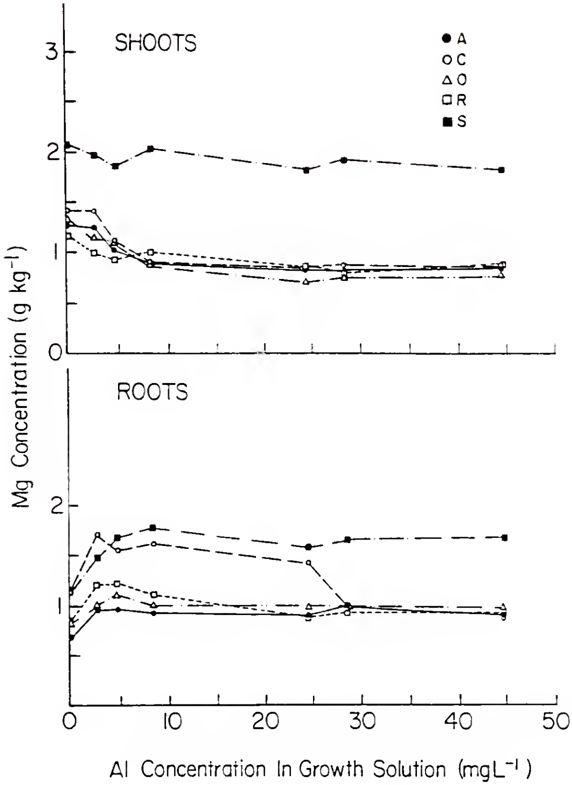

Figure 4-22. Magnesium concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

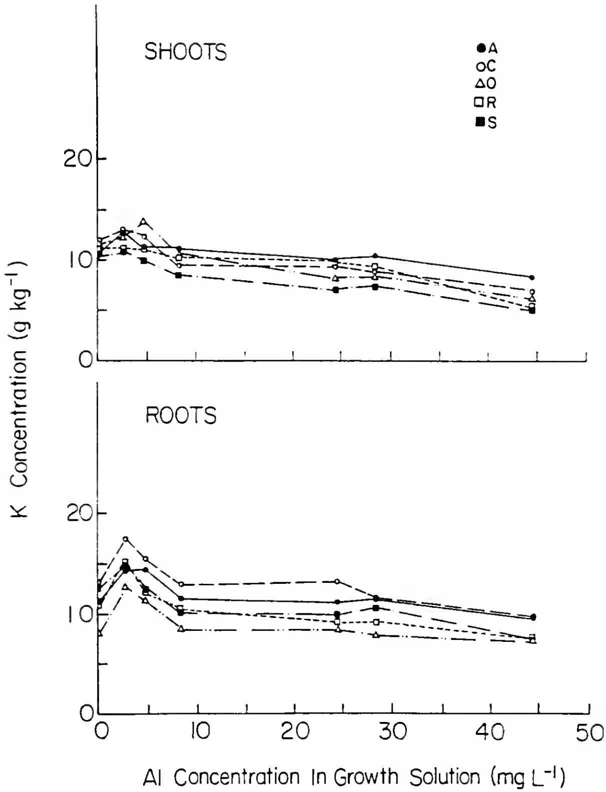

Figure 4-23. Potassium concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

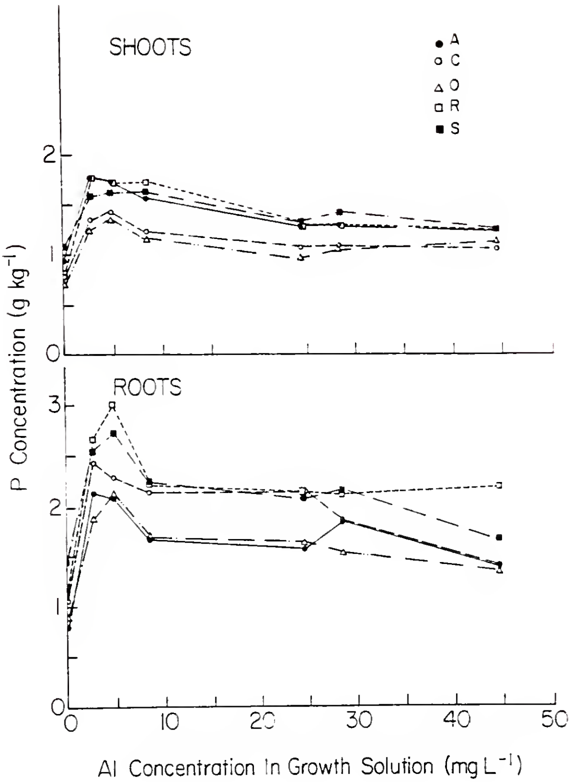

Figure 4-24. Phosphorus concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

concentrations then decreased. When Al concentrations were higher than 8.3 mg L^{-1}, the K and P concentrations decreased only slightly, however. At an Al concentration of 44.6 mg L^{-1}, P concentrations in both roots and shoots were still higher than those in the 0.1 mg Al L^{-1} treatment, while K concentrations were slightly lower. The K and P concentrations of roots and shoots were not related to Al-tolerance of the citrus rootstocks.

The mechanisms of beneficial effects of low Al concentration on plant growth generally have been associated with promoting P uptake (Mullette, 1975) or with correcting or preventing P toxicity (Clark, 1977). In the present study, there was no confounding effect of P on Al, because all treatments had nearly the same (adequate) P concentration in solution. The beneficial effects of Al were not caused by promotion of P uptake because Carrizo citrange, which roots had not been affected beneficially by Al at the 2.7 mg Al L^{-1} level, also evidenced increased P uptake. The beneficial effects were not caused by correcting or preventing P toxicity either, because there was no toxic level of P in the solution. Furthermore, the P concentration in roots of Carrizo citrange was lowest among the five rootstocks.

The mechanism of toxic effects of high Al concentration on plant growth have been ascribed to Al-induced P accumulation (McCormick and Borde, 1972) or deficiency (James et al., 1978). In this study, the highest P concentrations in roots or shoots at low Al treatments did not depress root or shoot growth. When root or shoot growth continuously decreased with increasing Al concentrations in solutions more concentrated than 8.3 mg L^{-1}, the P concentrations in the roots or shoots basically did not change. These data suggest that toxic

effects of Al were not caused by P accumulation. When plant growth continuously decreased, the P concentrations in the roots or shoots were still higher than those in the 0.1 mg Al L^{-1} treatment. Therefore, the toxic effects were not caused by P deficiency.

The fact that there was no certain relation between P concentrations in roots or shoots and Al-tolerance of citrus rootstocks also supports these explanations. It might be concluded that neither beneficial nor toxic effects of Al on growth of citrus seedlings were directly caused by P accumulation or deficiency induced by Al supply, although increased Al concentrations in the matrix caused an increase in P concentration of the plant tissues. The conclusions about P which have been made here could be applied to the cases of Mg and K in this study as well.

Zinc and manganese

The Zn and Mn concentrations in roots of the five rootstocks greatly decreased with increasing Al concentrations in solution up to 8.3 mg L^{-1} (Figs. 4-25 and 4-26). When Al concentrations in solution were higher than 8.3 mg L^{-1}, Zn and Mn concentrations in roots were maintained at the same levels or slightly decreased. However, the Zn and Mn concentrations in shoots basically did not change when Al concentrations in solution increased continuously from 0.1 to 44.6 mg L^{-1}. No certain relation was found between Zn and Mn concentrations in roots or shoots and Al-beneficial effects and Al-tolerance of citrus rootstocks.

Copper

In the 0.1 mg Al L^{-1} treatment, Carrizo citrange had the lowest Cu concentration in its roots among the five rootstocks. It seemed

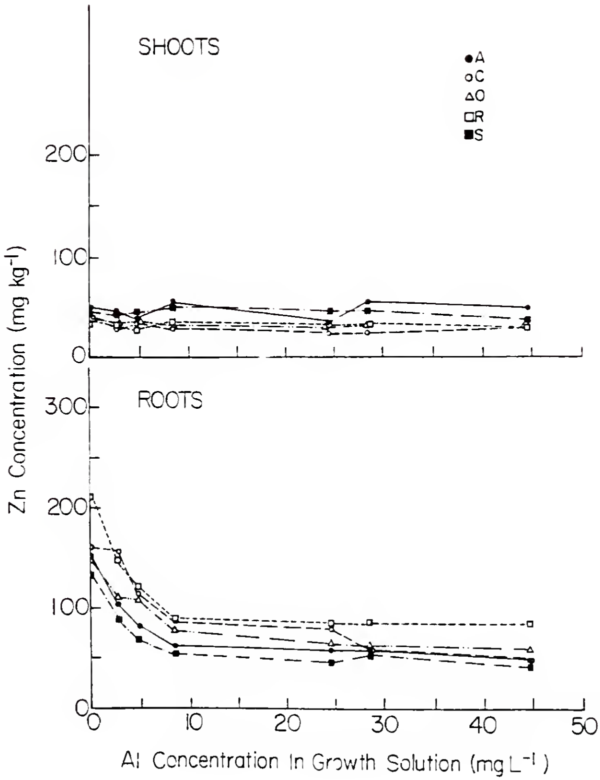

Figure 4-25. Zinc concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

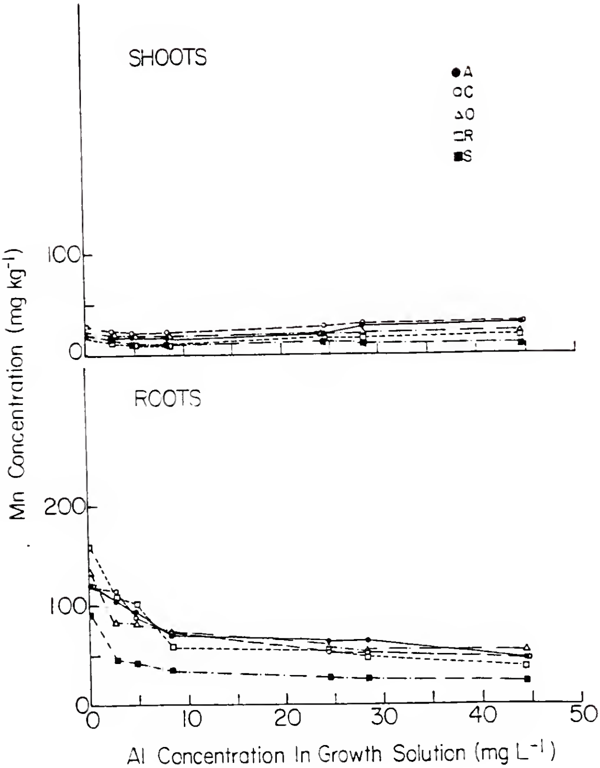

Figure 4-26. Manganese concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

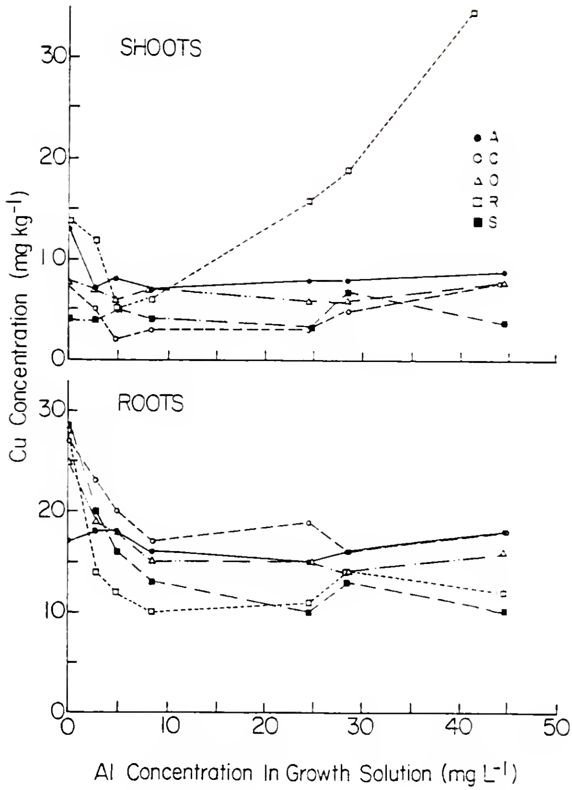

Figure 4-27. Copper concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

that the Al-sensitive rootstock had lower Cu concentrations in their roots than did the Al-tolerant rootstocks. The Cu concentrations in roots of all rootstocks except Carrizo citrange greatly decreased with increased Al concentrations in solution up to 8.3 mg L^{-1} (Fig. 4-27). Beyond 8.3 mg Al L^{-1}, the Cu concentrations in the roots changed only slightly. The Cu concentrations of Carrizo citrange changed only a little with increased Al concentrations in solution. The Cu concentrations in shoots decreased with increased Al concentrations in solution up to 2.7 or 4.8 mg L^{-1}, after those all rootstocks except Rough lemon changed little. The Cu concentrations in shoots of Rough lemon increased dramatically when Al concentrations in solution were higher than 4.8 mg L^{-1}. At the 44.6 mg Al L^{-1} treatment, the shoot Cu concentration of Rough lemon went up to 41 mg kg^{-1}, while the corresponding level for other rootstocks was only about 7 mg kg^{-1}. At this treatment, Al-toxic effect on shoot growth was largest for Rough lemon among the five rootstocks (Table 4-2). There seemed to be certain relation between Cu concentrations in roots and Al-tolerance of citrus rootstocks at 0.1 mg Al L^{-1} level in solution. Also, there seemed to be a relationship between Cu and the degrees of toxic effects of Al concentrations.

Iron

The Fe concentrations in roots greatly decreased with increased Al concentrations in solution up to 8.3 mg L^{-1} (Fig. 4-28). Beyond that Al concentration, the Fe concentrations in roots changed relatively little. When Al concentrations were lower than 8.3 mg L^{-1}, Rough lemon and Cleopatra mandarin had higher Fe concentrations than did the others, while Carrizo citrange had the lowest values

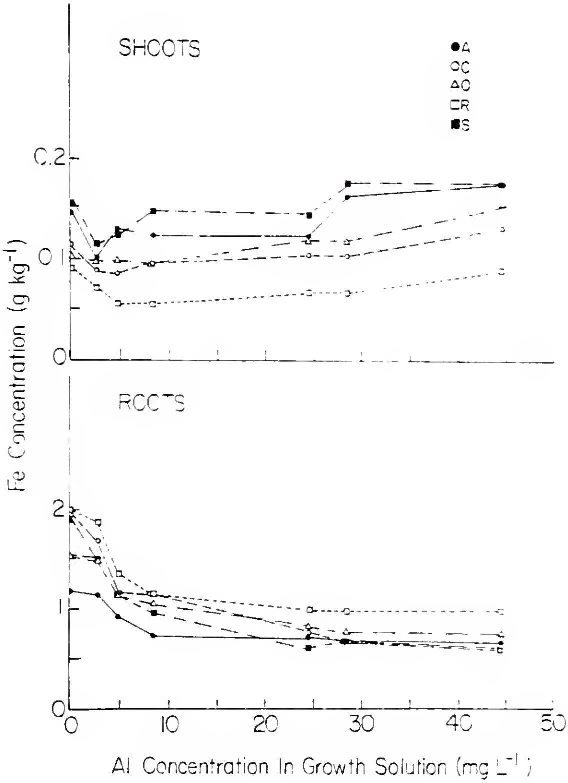

Figure 4-28. Iron concentration of 8-month-old citrus seedlings grown for 60 days in nutrient solution with various concentrations of Al. (A = Carrizo citrange; C = Cleopatra mandarin; O = Sour orange; R = Rough lemon; and S = Swingle citrumelo)

among the five rootstocks. By comparing this order and the Al-tolerance order of roots, suggestion might be made that the more tolerant rootstocks had the higher Fe concentrations in their roots when Al concentrations in solution were lower than 8.3 mg L^{-1}. The Fe concentrations in shoots decreased when Al concentrations in solution increased to 2.7 or 4.8 mg L^{-1}, however. Beyond this concentration the Fe concentrations in shoots increased only slightly. No relation between Fe concentrations in shoots and Al-beneficial effects and Al-tolerance of citrus rootstocks could be found.

Summary and Conclusions

Very few systematic studies have been conducted on the effects of Al on the growth and mineral nutrition of citrus. The objectives of this study were to investigate growth response of the most common citrus rootstocks in Florida to Al levels, and relations between the Al effects and elemental concentrations in plant tissue. Five 6-month-old citrus rootstock seedlings were grown in supernatant solutions which contained 7 levels of Al ranging from 0.1 to 44.6 mg Al L^{-1} and P concentration of 1 mg P L^{-1} for 60 days. The temperature of the growth solution was maintained at 25±1°C in the greenhouse during the summer. Before the seedlings were grown in solution, shoot height and fresh weight of whole plants were measured and the root length was measured by taking photographs of the roots for the purpose of later obtaining new-growth parameters. Results showed that, at high Al treatment levels, plants had thickened root tips and root caps covered with black gelatinous material. Unique Al injury

symptoms were observed in new leaves and terminals of some seedlings. The new-growth root length and shoot height had different trends with respect to response to Al concentrations in the growth solution. New-growth fresh weight of whole plants might be a better indicator for Al tolerance than the other two parameters mentioned above. According to the response of fresh weight to Al concentrations, relative aluminum tolerance of the rootstocks were Cleopatra mandarin > Rough lemon = Sour orange > Swingle citrumelo > Carrizo citrange. The critical Al concentrations in solution with respect to toxic effects were 12.2, 5.1, 5.1, 4.5 and 1.8 mg Al L^{-1}, respectively. Concentrations below or above the critical Al levels caused either beneficial or toxic effects, respectively. Aluminum concentrations of roots and shoots increased with increased Al concentration in the growth solution. Aluminum-tolerant rootstocks accumulated more Al in their roots than did the Al-sensitive rootstocks. When Al concentrations in nutrient solution increased from 0.1 to 4.8 mg Al L^{-1}, K, Mg, and P concentrations in roots and K and P levels in shoots increased; whereas Ca, Zn, Cu, Mn, and Fe in roots and Ca, Mg, Cu, and Fe in shoots decreased. It seemed that Al-sensitive rootstocks had lower Cu concentrations in their roots than did Al-tolerant rootstocks at low Al concentration (0.1 mg Al L^{-1}) in solution. The more tolerant rootstocks contained higher Fe concentrations in their roots than did the less tolerant ones when Al concentrations in solution were lower than 8.3 mg Al L^{-1}. Concentrations of the other elements (Ca, K, P, Mg, Zn, and Mn) in roots or shoots appeared to have no certain relationship with the beneficial or toxic effects of Al in nutrient solution, or with Al-tolerance of the rootstocks.

CHAPTER V

GROWTH OF CITRUS ROOTS AS AFFECTED BY ALUMINUM LEVEL IN SOILS UNDER FIELD CONDITIONS

Introduction

Research on the effects of Al on citrus rootstocks has been essentially limited to nutrient-solution studies. Haas (1936) used leafy-twig cuttings of lemon, Lisbon and Valencia oranges in solution cultures. He found that, when Al was present, roots were healthy and more extensive and root caps were numerous, but tops usually were retarded in growth. He concluded that "concentration of 15 to 20 ppm of aluminum was rather high for the production of the greatest growth" (of tops and roots). His data showed that the addition of Al to the culture solution also increased the P concentration in root tissue. Liebig et al. (1942) found that the addition of 2.5 to 5 mg Al L^{-1} to base nutrient solutions greatly stimulated root development but depressed top growth of Valencia orange and lemon cuttings. Lower concentrations (i.e., 0.1 and 0.5 mg L^{-1}) did not produce this effect. These researchers also found an antagonistic effect of Al on Cu uptake. Yokomizo and Ishihara (1973) concluded that root growth of Natsudaidai (*C. Natsudaidai* Hayata) seedlings in solution culture improved at low concentrations of Al but began to decrease when Al addition was 20 mg Al L^{-1}. Growth was extremely depressed at 100 mg Al L^{-1}.

Worku et al. (1982) found that high levels of Al and Mn were toxic to Troyer citrange [*Pencirus trifoliata* Raf. x *C. sinensis* (L) Osbeck], Trifoliate orange (*P. trifoliata* Raf.), and Cleopatra mandarin (*C. reshni* Hort. ex. Tan.) grown in highly weathered Oxisols. However, the growth-inhibiting effects of Al and Mn were considered jointly. Other researchers (Sekiya and Aoba, 1975; Huang, 1983) have linked low pH and high Al concentrations to poor citrus growth and shortened lifespan of the tree. However, no experimental evidence exists to evaluate the effects of different Al levels in soils on fibrous citrus-root growth under field conditions. Few data have been reported as well about possible effects of Al on the mineral nutrition of citrus.

The objective of this study was to use an implanted soil-mass technique (Garner and Telefair, 1954; Lund et al., 1970) to investigate the effects of different Al levels in soil on growth and mineral content of citrus fibrous roots under field conditions.

Materials and Methods

The implanted soil-mass technique allows one to study root development in a natural environment with minimal disturbance and minimal spatial and genetic variability.

This experiment was conducted using the implanted soil-mass technique in a commercial citrus grove.

Collection and Characterization of Soil

In order to get effects of high Al concentrations in soil solution when a certain amount of Al was added to the soil, the soil used for implanting must have low pH (pH < 5). The implanted soil

must also have low exchangeable Al content in order to obtain low Al concentration (<0.5 mg Al L^{-1}) in soil solution, so that non-treated soil could be taken as a control to get critical Al concentration in soil solution for phytotoxicity.

Soil used for implanting was obtained from the E horizon of an Immokalee fine sand (an Arenic Haplaquod) from a citrus grove in the "flatwoods" area of Charlotte County, Florida. The overlying Ap horizon was first removed by hand-shoveling before collecting the bulk sample of E horizon. The soil was air-dried and passed through a 2-mm sieve.

Soil pH was measured with a 1:1 water:soil ratio. Particle-size analysis was conducted using a pipette sampling method (Soil Conservation Service, 1972). Soil organic C was determined by a modified Walkley-Black procedure (Nelson and Sommers, 1982). Potassium-chloride extractable acidity, exchangeable Al, and exchangeable H were determined in 1 M KCl extracts (Thomas, 1982). Effective CEC of soil was calculated from the sum of exchangeable bases by 1 M NH_4OAc (pH 7.0) (Chapman, 1965) and exchangeable Al (1 M KCl). Calcium, Mg, K, Na, P, Al, Zn, Fe, Cu, and Mn were extracted with double-acid reagent, 0.05 M HCl and 0.025 M H_2SO_4 (Mehlich, 1955), and determined by inductively-coupled argon plasma (ICAP) emission spectroscopy. Relevant characteristics of the soil are listed in Table 5-1. It had a high sand content; was strongly acidic; and had a low exchangeable-Al content.

Addition of Lime, Al, and Fertilizers

There were five treatments (addition of lime and addition of 0, 15, 18, or 24 mg Al kg^{-1} of soil). A loosely woven mesh-saran bag

Table 5-1. Relevant characteristics of the E horizon of the Immokalee fine sand used for implants.

Soil property	
pH	4.20
Organic C, g kg^{-1}	0.6
Particle size	
Sand, %	97.5
Silt	1.2
Clay	1.3
ECEC, $mmol_c$ kg^{-1}	1.31
1 *M* KCl extractable acidity	
Total, $mmol_c$ kg^{-1}	0.62
Al	0.11
H	0.51
0.05 *M* HCl and 0.025 *M* H_2SO_4 extractable elements	
Ca, mg kg^{-1}	27.9
Mg	3.4
K	3.0
P	0.4
Na	1.2
Al	1.5
Fe	2.4
Zn	0.1
Cu	0.3
Mn	0.1

(hole size 3 x 2 mm) was used to hold 4.5 kg of E-horizon soil. The limed soil was amended with 125 mg chemically pure $CaCO_3$ kg^{-1}, the amount of lime required to bring soil pH to 6.5 as specified by the Adams-Evans method (McLean, 1982). The Al was added as solutions of $AlCl_3 \cdot 6H_2O$.

Blanket fertilizer additions in solution form also were made to the soil in each bag. The fertilizer program recommended by Koo et al. (1984) was taken as a reference. Fertilizer rates (mg kg^{-1}) and forms were as follows: 5.93 N as $Ca(NO_3)_2 \cdot 4H_2O$; 0.89 P as $Ca(H_2PO_4)_2 \cdot H_2O$; 9.10 Ca as $Ca(NO_3)_2 \cdot 4H_2O$ + $Ca(H_2PO_4)_2 \cdot H_2O$; 5.35 K as KCl; 3.88 Zn as $ZnSO_4 \cdot 7H_2O$; 1.20 Mg as $MgCl_2 \cdot 6H_2O$; 0.13 Fe as $FeSO_4 \cdot 7H_2O$; 0.22 Mn as $MnSO_4 \cdot H_2O$; 0.13 Cu as $CuSO_4 \cdot 5H_2O$; and 0.01 B as H_3BO_3. After the lime or Al solution and fertilizer solution had been added, the soil in each bag was mixed thoroughly and the moisture level was adjusted to 12%. The soil then was allowed to equilibrate with the amendments for 18 d at room temperature in the laboratory.

Placement and Collection of Implanted Soil-Mass Bags

A typical commercial citrus grove (with 30-yr-old trees of *C. sinensis*, cv. Hamlin/*C. aurantium* L. sour orange rootstock) in De Soto County, Florida was selected for the study. The experiment was conducted according to a randomized complete-block design, with five treatments assigned randomly in each of 15 blocks. Fifteen healthy-appearing trees were marked as blocks. Five holes (20-cm deep and 16-cm diameter) were dug at the dripline of each tree (about 3 m from the tree trunk) with a post-hole digger. The exact location of each hole used for implant was determined by first digging a hole, screening out the roots on-site, and comparing the quantity of roots to

prescribed limits (about 8 roots). A bag containing the implant soil then was placed firmly against the face of the hole on the side toward the tree trunk. Some original top soil was tamped firmly around each bag, with the bag then being left open at the soil surface to approximate the same field conditions as the surrounding surface soil. All of the holes were dug, and all of the bags were installed in the holes, on 2 August 1988. The areas under the trees and within 2 m of the holes were kept free of understory vegetation, to minimize invasion of the bags by non-citrus roots.

All bags were removed 46 d after their insertion. The roots around the outside of the bags were cut off with a long knife and then the bags were removed from the holes. Each bag was trimmed of protruding roots and placed in separate plastic bags to prevent soil and moisture loss during transport to the laboratory in Gainesville, Florida.

Measurement of Roots and Analysis of Implanted Soils

After removal from the bag, the roots separated from the soils were put on a screen and then washed thoroughly with tap water and rinsed with deionized water. Root morphology was assessed visually and root length was measured directly. Root length is a better indicator of Al effects than is root weight, since shortening and thickening are common results of Al treatment (Munn and McCollum, 1976). Roots which entered the bag from its surroundings were classified as primary roots. All branches produced from these primary roots were classified as secondary.

All roots were dried at 70°C and weighed. For tissue analysis, because of the small quantities recovered per bag, the roots of the

15 replications of a given treatment were randomly combined into 3 samples. The roots were ground in a mortar to pass a 20-mesh sieve. Tissue samples of 0.2 g were dry-ashed at 500°C in a muffle furnace for 4 h; the ash was then dissolved in 10 mL of concentrated HCl, evaporated to dryness, redissolved, and evaporated to dryness again. This residue was dissolved in 10 mL of 0.1 M HCl and filtered. Elemental contents in the solution were determined using ICAP emission spectroscopy.

Just before the amended soil was placed in the grove, and again after collection, the concentrations of Al and of other elements in saturation soil extracts (Rhoades, 1982) were determined using ICAP emission spectroscopy. Electrical conductivity (EC_e) and pH were measured immediately after extraction. Values before implanting and after collection were averaged to represent the implanted period (46 d).

Results and Discussion

Selected characteristics of soil saturation extracts are shown in Table 5-2. When 1.0 kg soil was treated with 15 mg Al, Al concentration and EC_e sharply increased and pH decreased relative to the control. As the amount of added Al increased from 15 to 24 mg Al kg^{-1}, Al concentration increased greatly while pH decreased and EC_e increased only slightly. Aluminum concentration decreased and pH increased in lime-amended soil.

At the time of bag removal, we observed some roots which had grown downward in soil outside but adjacent to the bag's outer surface, apparently avoiding entry into the soil inside the bag.

Table 5-2. Relevant characteristics of soils from five treatments.

Treatment	Al added to soil	Saturation extract of soils† Al Before implant	After implant	Average†	EC_e†	pH†
	$mg\ kg^{-1}$	----------$mg\ L^{-1}$		-----------	$dS\ m^{-1}$	
Al-0	0	0.21	0.05	0.13 d‡	0.30 c	5.1 b
Al-1	15	15.94	2.34	9.14 c	0.71 b	3.7 c
Al-2	18	37.72	5.46	21.59 b	0.83 ab	3.5 c
Al-3	24	59.63	9.57	34.60 a	0.99 a	3.4 c
Lime	0	0.03	0.02	0.03 e	0.32 c	6.4 a

† Each value is the average of 2 means, i.e., mean values just before implanting or after collection for 15 replications.

‡ Values followed by the same letter in a column are not significantly different at P = 0.05 by Duncan's multiple-range test.

Roots in the soils of the Al-1 and Al-2 treatments appeared healthier, coarser and firmer than those in the control soil. There were also more secondary roots. These roots were white in color, whereas roots in the Al-3 treatment displayed abnormal root symptoms; i.e. they were retarded, stubby and brittle. There were also fewer secondary roots, the root tips became thickened, and some of them turned brown.

Average root-length densities (cm root length per dm^3 soil) are given in Table 5-3. Root-length density for the Al-1 treatment (9.14 mg Al L^{-1}) was significantly higher than that for the control (0.13 mg Al L^{-1}). Root-length density for treatment Al-2 (21.59 mg Al L^{-1}) was lower than that for treatment Al-1 but showed a tendency to be higher than that for the control. Root-length density for the Al-3 treatment (34.60 mg Al L^{-1}) was significantly lower than that for the control. Factors known to affect Al phytotoxicity include temperature, pH, organic matter, and soil solution concentrations of Al, Ca, Mg, and P (Rhue and Grogan, 1976). All treatments were in the same thermal environment, with air temperatures during the 46 d ranging from 21 to 37°C, and averaging 27°C. The pH values of Al-amended soils were lower than that of the control (pH 5.1), being less than pH 4 (Table 5-2). Since a large portion of the added Al was in soluble form, the effect of Al should be much larger than that of pH. Also, the pH values were similar (3.7, 3.5 and 3.4) across the three Al treatments. Therefore, pH of the Al-treated soils was probably not the main cause of the significant differences in root densities.

The EC_e values ranged from 0.30 to 0.99 dS m^{-1} (Table 5-2). Citrus root growth should not be significantly affected by EC_e levels

Table 5-3. Fibrous citrus-root growth in implant bags of soil (3.17 dm^3) after 46 d as related to treatments.

Treatment	Primary roots		Secondary roots			Total root-length density‡
	Number	Length	Number	Length	Root density†	
	Roots bag^{-1}	cm $root^{-1}$	Roots bag^{-1}	cm $root^{-1}$	Roots cm^{-1}	cm dm^{-3}
Control	9.6 b§	3.9 b	6.9 c	1.3 b	0.22 b	14.7 b
Al-1	15.8 a	4.0 b	23.8 a	1.4 b	0.33 a	30.6 a
Al-2	11.0 ab	3.8 b	13.7 b	1.0 b	0.35 a	18.3 b
Al-3	5.8 c	3.1 c	3.3 d	1.9 a	0.20 b	8.3 c
Lime	13.4 a	4.8 a	16.6 b	1.2 b	0.25 ab	28.4 a

† Average secondary root density is the number of secondary roots per unit length of primary roots.

‡ Average root-length density is the total length of primary, secondary, and tertiary roots per unit volume of soils.

§ Means followed by the same letter in a column are not significantly different at $P = 0.05$ by Duncan's multiple-range test.

in this range (Boaz, 1978). The organic matter content of the surrounding soil was also low (Table 5-1). All of the important elements except Al however, were assumed to be in adequate supply because the same amounts of nutrients were applied to all treatments. For the Al-treated soils, concentrations of Ca, Mg, and P in soil saturation extracts were quite similar, ranging from 73 to 80, 11 to 12, and 0.5 to 0.6 mg L^{-1}, respectively. Thus, concentrations of these elements should not have significantly influenced the effects of Al on root growth. Rather, Al supply per se appeared to be the main cause of increases or decreases in root density for the Al-amended soils.

The fraction of soil Al in the soil solution directly affects root growth. In the present study, both the ECEC and the exchangeable Al level of the soil were low. The organic C content of the soil was so low that the organic-matter-bound content of Al would have been small. Thus, under the conditions of this study, a large portion of the added Al remained in the soil solution, as evidenced by Al in the saturated soil extracts (Table 5-2). Aluminum in the saturation extract of soils should be closely related to citrus root growth.

Efforts to relate phytotoxicity to a certain species of Al in the soil solution have met with variable degrees of success. There is no clear consensus on which species (Al^{3+}, hydroxy-Al monomers, or hydroxy-Al polymers) dominates phytotoxicity (Alva et al., 1986; Parker et al., 1989). In other studies, root growth in acid soils was significantly related to total Al concentration in the soil solution (Ragland and Coleman, 1959; Adams and Lund, 1966; Brenes and

Pearson, 1973; Pavan et al., 1982; Wright et al., 1987). In the present study, organic matter content of the soils was low and the range in pH of the Al-treated soils was narrow (3.4 to 3.7). In addition, saturation extracts of Al-treated soil had similar concentrations of SO_4^{2-} and F^- which seem to ameliorate toxicity by complexing Al (Cameron et al., 1986; Kinraide and Parker, 1987). Thus, concentrations of Al^{3+} or hydroxy-Al should be highly correlated with total Al concentration. Therefore, no matter what species of Al dominates phytotoxicity, the total Al concentrations in soil saturation extracts should have provided a good relative indicator of levels of toxic Al species that potentially affect citrus root growth in this study.

Effects of Al concentrations in soil saturation extracts on fibrous root-length density are shown in Fig. 5-1. Aluminum concentration in the control was so low [0.13 mg L^{-1}, i.e., $(Al)^{1/2} = 0.36$] that this concentration would not produce any beneficial or toxic effect on citrus root growth (Liebig et al., 1942). Therefore, the root-length density of this treatment was taken as a control. When Al concentration was 9.1 mg L^{-1}, the root-length density was almost twice as high as that for the control. When Al concentration increased to 21.6 mg L^{-1}, the root-length density decreased but was still somewhat higher than that for the control. However, when Al concentration increased to 34.6 mg L^{-1}, root-length density decreased to about 65% of the value for the control. The Al concentration at which the root-length density was equal to that of the control was considered a critical Al concentration. In order to get the critical value, a regression equation was calculated. Between the control and

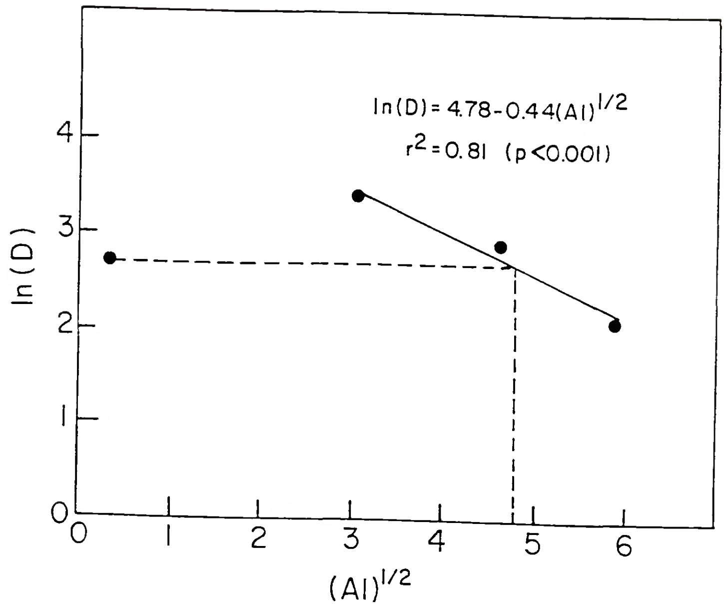

Fig. 5-1. Effects of Al concentration (Al, mg L^{-1}) in soil saturation extracts on fibrous root-length density (D, cm dm^{-3}). Critical Al concentration was 23 mg Al L^{-1} [i.e., $(Al)^{1/2}$ = 4.8].

Al-1 treatment, root-length densities increased but the curve was uncertain. The root-length densities gradually decreased from the Al-1 to the Al-3 treatment; thus, the regression equation was based on three treatments (A-1, A-2, and A-3). From Fig. 5-1, the critical Al value appears to be 23 mg L^{-1} [i.e., $(Al)^{\frac{1}{2}}$ = 4.8]. For mature citrus trees, when Al concentration in the saturation extract was lower than 23 mg L^{-1}, Al had a beneficial effect on fibrous root growth; higher Al concentrations decreased root growth. Thaworuwong and Van Diest (1974) found that young seedlings were generally more susceptible to Al toxicity than were older plants.

The beneficial effects of the Al-1 and Al-2 treatments were expressed as greater numbers of both primary and secondary roots, and increased average secondary-root density (Table 5-3). These facts indicate that more roots entered the bag to grow in the environment inside the bag and that more lateral roots were produced. It is unknown whether Al-induced root proliferation might benefit the harvestable part of the plant. The average length of primary and secondary roots did not increase, however. The adverse effects of the Al-3 treatment were expressed as fewer primary and secondary roots, and a smaller average length of primary roots. Thus, fewer roots grew into the bag from the surrounding environment, and they produced fewer lateral roots. These primary roots also were shorter than those of the control.

The roots in lime-amended soils appeared to be more vigorous and healthier than those of the control treatment, and root-length density was significantly higher (Table 5-3). The increased root-length density due to liming arose both from the increased number of

primary and secondary roots, and increased average length of primary roots. Liming also produced more tertiary roots. The Al concentrations in saturation extracts of lime-amended soil were lower than for the control (Table 5-2). Therefore, the aluminum concentrations in soil solutions of lime-amended soil probably were not the main cause of increased root-length density. Some authors have concluded that citrus grows better near neutrality than under acid conditions (Haas, 1936; Worku et al., 1982; Koo et al., 1984). The pH of the lime-amended soil had been raised to 6.4, while the control soil was 5.1. Thus the increased pH could be the main cause of increased root-length density.

When root growth of the Al-1 treatment was compared with the lime treatment, it showed that the beneficial effect of Al on fibrous citrus-root growth could be as large as that of liming. The results of this study suggest that, when the active Al level in an acid soil is below a certain level (e.g., about 23 mg Al kg^{-1} in the saturated soil extracts in this study), the beneficial effect of lime is not caused by reduction of Al activity. Instead, lime may have eliminated inhibitory factors related to soil pH, or corrected Ca or Mg deficiencies, or improved availability of other nutrients.

The Al concentrations of roots in the Al-1, Al-2, and lime treatments were lower than those in the control, while the Al concentrations for the Al-3 treatment were higher (Table 5-4). A possible explanation for the lower concentrations is that the increased Al or increased pH in the soil greatly increased root dry matter, thereby causing a dilution of Al concentrations in the root tissues (Munson and Nelson, 1973). Total root uptake of Al per bag in the Al-1,

Table 5-4. Concentrations of elements in citrus fibrous roots.

Treatment	Ca	Mg	K	P	Na	Al	Fe	Zn	Cu	Mn	B
	$g\ kg^{-1}$				$mg\ kg^{-1}$						
Control	6.8 ab†	2.8 b	10.1 b	1.5 a	1313 a	424 b	2008 a	193 a	79 a	70 a	25 ab
Al-1	7.3 a	3.2 ab	10.8 b	1.5 a	731 b	352 d	541 b	71 c	43 c	26 c	22 b
Al-2	6.4 b	3.2 ab	9.9 bc	1.5 a	774 b	406 c	250 c	57 d	40 c	27 c	23 b
Al-3	6.8 ab	3.3 a	8.9 c	1.4 a	1253 a	446 a	205 c	65 cd	65 b	40 b	28 a
Lime	7.3 a	3.0 ab	11.7 a	1.6 a	802 b	356 d	479 b	248 b	63 b	43 b	22 b

† Means followed by the same letter in a column are not significantly different at $P = 0.05$ by Duncan's multiple-range test.

Al-2, and lime treatments was much higher than that for the control. Mechanisms for the beneficial effects of Al are not yet fully understood (Foy, 1984). The concentrations of Na and Cu in roots showed trends that were similar to that of Al. In general, the concentrations of Zn, Fe, and Mn in roots decreased with increased additional Al in the soil. However, the concentrations of Ca, Mg, K, P, and B in roots were similar for all five treatments. Data obtained by Yokomizo and Ishihara (1973) showed the same trends for Zn, Mn, Ca, Mg, and P in Natsudaidai roots. The concentration changes for some elements in roots were not simply due to concentration changes in the soil solution, because each of the elements listed in Table 5-4 (except Al) had almost the same concentrations in Al-treated soil solutions.

Summary and Conclusions

Research on the effects of Al on citrus rootstocks has been essentially limited to nutrient-solution studies. This study was conducted with an implanted soil-mass technique to investigate the effects of Al level in soils on growth and mineral content of fibrous citrus roots under field conditions. The implanted soil, E horizon material from an Immokalee fine sand (an Arenic Haplaquod), had a pH of 4.2 and a very low exchangeable-Al content. It was either non-amended (control), or amended with either lime or three levels of Al using $AlCl_3$. Each of the five treatments was replicated fifteen times. Mesh bags containing the amended soil were placed in holes at the dripline of mature trees in a commercial citrus grove. The bags were removed after 46 d. Results indicated that, when Al concentration in the saturation extract of soils was below 23 mg L^{-1}, Al had a

beneficial effect on growth of fibrous roots of _C. aurantium_ L. sour orange rootstock. At a concentration of 9.1 mg Al L^{-1}, root density was almost twice that of the control (0.1 mg Al L^{-1}), and equalled that of the lime treatment. More roots grew into the bag, and they produced more lateral roots. Aluminum concentrations in roots were lower, however, than those in the control. Root growth retardation occurred only when Al in the saturation soil extract exceeded 23 mg L^{-1}. In general, the concentrations of Zn, Fe, and Mn in roots decreased with increased Al application to the soil, while concentrations of Ca, Mg, K, P and B were unchanged.

CHAPTER VI

EFFECTS OF LIME AND PHOSPHOGYPSUM ON FIBROUS CITRUS-ROOT GROWTH AND PROPERTIES OF THE Bh HORIZON OF A SPODOSOL

Introduction

Thousands of hectares of citrus on the central Florida ridge have been eliminated due to recent severe freezes. Therefore, South and Central Florida Spodosols are being used increasingly for citrus production. The depth of soil available for root development of citrus trees is much less in the "flatwoods" Spodosols than in the Florida ridge Quartzipsamments (D.L. Myhre, unpublished data). Because of this reduced rooting depth, fruit yields per tree and per hectare are generally about 40 percent lower on the "flatwoods" soils than on the ridge soils (Calvert, 1979).

Spodosols in the Southern Florida Flatwoods Land Resource Area (Caldwell, 1980) are generally acidic (pH < 5 in the spodic horizon). The Al concentration in the soil solution increases with a decrease in pH so that, at pH < 5.0, Al concentrations are generally toxic to plants (Kamprath, 1980). Several researchers (Haas, 1936; Liebig et al., 1942; Yokomizo and Ishihara, 1973) found that high Al concentrations were toxic to citrus root growth. Other researchers (Sekiya and Aoba, 1975; Huang, 1983) have also linked low pH and high Al concentrations to poor citrus growth and shortened lifespan of the tree.

The reduced rooting depth in the "flatwoods" soils is most likely due to high water tables, and perhaps to acidity in those portions of the spodic horizons which are kept above the water table by artificial drainage. It is possible that Al toxicity may be one of the factors inhibiting citrus-root growth in Bh-horizon soil. If the chemical environment in the subsoil of these Spodosols can be made more favorable for root growth, soil rooting volume and fruit yield can be increased.

The effects of liming are primarily limited to the Ap horizon of many Spodosols (Calvert et al., 1978). Acidity in the subsoil is difficult to alleviate, due in part to the difficulty of lime placement at lower soil depths (Pearson, 1966). Calvert et al. (1978) modified an acidic Spodosol by applying 56 Mg dolomitic lime ha^{-1} before deep-mixing of the soil to a depth of 107 cm. This treatment was responsible for significant changes in both physical and chemical properties of the mixed soil (Fiskell and Calvert, 1975). Liming deep-tilled plots resulted in doubling of the cation exchange capacity in the 90-cm profile compared to deep-tillage alone.

Phosphogypsum (PG) is stockpiled in large quantities throughout Central and North Florida. If PG could be used as an ameliorant for the subsoil acidity syndrome, some citrus areas could benefit from application of this amendment. The use of a state-industry "waste-product" would also afford that industry a means of disposing of a material otherwise unwanted. One experiment was initiated in 1964 in a 3-yr old grove on upland "ridge" soil (Anderson, 1968). The high rate of PG application (33.3 Mg ha^{-1} over 2.5 yr) reduced tree growth, cold tolerance, and fruit yields. Results of a recent

column-leaching study (Oates and Caldwell, 1985) showed that substantial amounts of exchangeable Al can be removed from an acidic (pH 4.9) PG-amended subsoil if adequate leaching occurs. Soybeans grown on this amended soil after leaching had significantly higher plant weights than soybeans grown on non-leached, amended soil. No experimental data exist, however, for recommendation of soil application of PG to "flatwoods" soils used for citrus.

Since it is impractical to apply amendments directly to the Bh horizon in-place, it would be better for research purposes to place some Bh-horizon soil in the surface horizon where the effects of the amendments on root growth can be measured. The implanted soil-mass technique used in Chapter V of this dissertation should be suitable for this purpose.

The objectives of this study were to utilize the implanted soil-mass technique to assess fibrous citrus-root growth in spodic horizon soil amended with lime and PG, and to measure changes in Al status and other properties of soils due to the lime and PG amendments under field conditions.

Materials and Methods

This experiment was conducted with the implanted soil-mass technique in a citrus grove.

Characterization of Bh-horizon Soil

The soil used as implanted mass in this investigation was obtained from the Bh horizon of a Smyrna fine sand (Aeric Haplaquod) from a citrus grove in the "flatwoods" area of DeSoto County, Florida. The soil was taken from the Bh horizon after first removing the

over-lying Ap and E horizons. The soil was kept in a moist state and passed through a 6-mm sieve.

Soil pH was measured at a 2:1 water:soil ratio. Calcium, Mg, K, P, Na, Fe, Zn, Cu, and Mn were extracted with 0.05 M HCl and 0.025 M H_2SO_4 (Mehlich, 1955), and the quantities in the extracts were determined with inductively coupled argon plasma (ICAP) emission spectroscopy. Soil organic matter was determined by a modified Walkley-Black procedure (Nelson and Sommers, 1982). Potassium chloride-extractable acidity (exchangeable Al and exchangeable H) was determined by a 1 M KCl method (Thomas, 1982). The selected characteristics of the soil are listed in Table 6-1. The soil was strongly acidic and had a much higher exchangeable-Al content than exchangeable-H content.

Application of Amendments: Lime and PG were used as amendments to the Bh-horizon soil. The pH of the PG was 4.5. The PG consisted of 22% Ca, 16% S, 0.3% P, 0.5% F, and 0.07% Al, with the remainder being bound oxygen, free water, and impurities. A loosely-woven mesh-saran bag was used to hold the implanted Bh-horizon soil. Each bag of soil weighed about 4.2 kg. Each treatment (control, lime-amended soil, and PG-amended soil) had 40 bags of soil. The limed soil was amended with 17.3 g $CaCO_3$ per bag, which was the amount of lime needed to bring the soil pH to 6.5 as determined by the Adams-Evans buffer lime-requirement test (Adams and Evans, 1962). The soil with the PG treatment received a quantity of PG equal to five times the number of Al equivalents kg^{-1} extracted from the soil by 1 M KCl. The amount of PG added was 21.8 g per bag. Soil with or without the

Table 6-1. Selected chemical characteristics for the Bh horizon of the Smyrna fine sand used for implants.

Soil property	
pH	4.57
Organic C, g kg^{-1}	23.5
1 M KCl extractable acidity	
Total, $cmol_c$ kg^{-1}	1.58
Al	1.38
H	0.20
0.05 M HCl and 0.025 M H_2SO_4 extractable elements	
Ca, mg kg^{-1}	316
Mg	84
K	33
P	141
Na	30
Fe	3.6
Zn	3.3
Cu	0.0
Mn	1.0

amendment in each bag was mixed thoroughly, and distilled water was added to each bag periodically to keep the soil-moisture level at approximately 10%. The soil was allowed to equilibrate with the amendments for 30 d in the laboratory.

Placement and Collection of Implanted Soil-Mass Bags

A typical citrus grove with 30-yr old trees Hamlin [*C. sinenis* (L.) Osbeck]/Sour orange [*C. aurantium* (L.)] in DeSoto County, Florida was selected for the study. The experiment was conducted according to a split-plot design with a randomized complete-block arrangement of the whole-plot factor (four sampling dates). The sub-plot factor was three treatments (control, lime-amended soil, and PG-amended soil). Forty healthy-appearing trees were selected and divided into 10 blocks. Each of four trees in each block was selected for each of four sampling dates. Three holes (20-cm deep and 15-cm diameter) were dug near each tree with a post-hole digger, one for each of three treatments. Each of the three holes was about 1 m apart and about 3 m from the tree trunk. A bag containing the soil was placed in each hole. The bag was placed firmly against the face of the hole on the side toward the tree trunk. Some original top soil removed from the hole was tamped firmly around the bag. The bags were left open at the soil surface to allow water movement into and through the soil in the bag. All of the holes were dug, and all of the bags were installed in the holes, on 16 May 1987 (zero d). The areas under the trees and within 2 m of the holes were kept free of any understory vegetation to minimize invasion of the soil bags by non-citrus roots. The four bag-removal dates were 10 July (55 d),

8 August (84 d), 6 September (113 d), and 1 October (139 d). The roots around the outside of the bags were cut off with a long knife and then the bags were removed from the holes. Each bag was trimmed of protruding roots, and samples were then placed in separate plastic bags to prevent soil loss during transport.

Measurement of Roots and Analysis of Implanted Soil

After each sampling, the root-length density (cm root cm^{-3} soil) for each bag was measured by the line-intersect method (Tennant, 1975), and total root weight was determined. Soil pH, KCl-extractable acidity, exchangeable Al, and exchangeable H were determined as they had been at the start of the experiment. Electrical conductivity (EC_e) in the saturation extract (Rhoades, 1982), $BaCl_2$-TEA (pH 8.2) exchange total acidity (Thomas, 1982), and ions in the saturation extract (Rhoades, 1982) were also determined. For the fourth sampling, soil CEC by 1 M NH_4OAc (pH 7.0) was determined as well (Chapman, 1965). Soluble Ca^{2+} from the lime and PG were high, resulting in much higher exchangeable Ca^{2+} contents in the lime- and PG-amended soil than in the control. Therefore, the effective cation exchange capacity, i.e., the sum of exchangeable Al (1 M KCl) and exchangeable bases, and CEC as the sum of exchange total acidity ($BaCl_2$-TEA, pH 8.2) and exchangeable bases, were not reliable (data not shown). The values of CEC determined by 1 M NH_4OAc (pH 7.0) are presented although there may be error due to the competition of Ca^{2+} with NH_4^+ for some exchange sites on the soil.

Results and Discussion

Fibrous-Root Growth in the Bags of Soil

The mean root-length densities of three treatments for each of four samplings are shown in Fig. 6-1. The density variations for the control or PG-amended soils among four sampling dates might be due to variations in physiological root growth during different periods and also to variations in environmental conditions, such as rainfall and temperature. The orthogonal contrasts of the mean root-length densities are listed in Table 6-2. The analysis of root weight showed the same results as root-length density, because there was a highly significant, positive, correlation between root weight and root length ($r^2 = 0.90$, $P < 0.001$) (Table A-3). No significant difference occurred between the control and the lime-amended soil at the first bag-removal date. However, for the next three dates, lime-amended soil had higher mean-root densities than the control. In general, the lime amendment significantly improved root growth. At the first date, the root-length density of PG-amended soil was significantly lower than that of the control. At the second and fourth dates, they were similar to each other. At the third date, the root density of PG-amended soil was significantly higher than that of the control. During the four root-growth periods, mean daily rainfall averaged 4.42, 4.34, 6.74, and 4.38 mm, respectively (Table A-4). The high mean rainfall of the third growth period might have caused higher root-length density for the PG-amended soil because of the Al leaching effect (Oates and Caldwell, 1985). In general, the PG-amendment did not have any detrimental or beneficial effect on fibrous citrus-root growth.

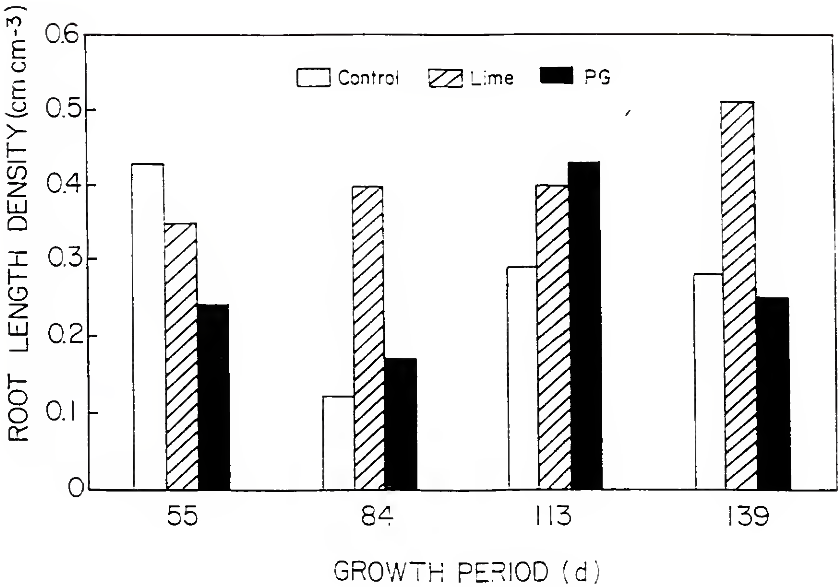

Figure 6-1. Mean root-length densities for three treatments at four sampling periods.

Table 6-2. Contrasts for root-length density as affected by lime and phosphogypsum (PG) amendments to the Bh horizon of a Smyrna fine sand.

Sampling time	Contrast of mean root-length density	
	Control vs lime	Control vs PG
	PROB > F	
1st	0.37	0.04
2nd	0.07	0.74
3rd	0.11	0.05
4th	0.08	0.77
Overall samples of four dates	0.01	0.85

Selected Chemical Properties of the Soils

The values of pH, EC_e, KCl-extractable acidity, exchangeable Al, exchangeable H, and $BaCl_2$-TEA exchange total acidity for each treatment were similar (i.e., not significantly different, $P > 0.05$) for each of the four harvest dates (Table A-5). The contrasts of these properties between control and lime-amended soil, and between control and PG-amended soil, also essentially remained the same for each of the four harvest dates. The soil-property means for the four dates, along with the contrasts between them, are listed in Table 6-3. The EC_e values of the lime-amended soil and of the control were similar, while the EC_e of the PG-amended soil was significantly higher ($P < 0.01$) than that of the control. The high EC_e of the PG-amended soil might have been due to the high soluble-Ca and -sulfate contents of PG, which added 1.16 g Ca and 2.61 g SO_4^{2-} to each kg of soil. According to Boaz (1978), citrus-root growth is not significantly affected by EC_e levels up to 2.2 dS m^{-1}.

The values for CEC determined by 1 $\underline{M}$ NH_4OAc (pH 7.0) were not significantly different among the three treatments. Application of lime raised soil pH from 4.6 to 6.3 and significantly reduced exchangeable-Al content, exchangeable-H content, KCl-extractable acidity, $BaCl_2$-TEA exchange total acidity and Al saturation. These improved conditions appeared to be favorable to fibrous citrus-root growth. The exchangeable-Al content in lime-amended soil was much less than that in the control. This reduction in exchangeable-Al content probably caused higher root density in lime-amended soil than in the control. The PG-amended soil had lower pH and higher

Table 6-3. Contrasts of selected chemical properties of the soils (four dates).

	Mean (n = 40)			PROB > F	
	Control	Lime	PG	Control vs Lime	Control vs PG
pH	5.2	6.4	4.7	<0.01	<0.01
EC_e ($dS\ m^{-1}$)	0.4	0.3	2.2	0.26	<0.01
	---- $cmol_c\ kg^{-1}$ ----				
CEC†	6.89	6.62	7.03	0.12	0.43
KCl-extractable‡ acidity	0.85	0.08	0.93	<0.01	0.14
Exch. Al‡	0.68	0.08	0.78	<0.01	0.04
Exch. H‡	0.17	0	0.15	<0.01	0.56
$BaCl_2$-TEA exch. total acidity	11.12	6.22	11.44	<0.01	0.06
	----------%----------				
Al saturation†	9.6	1.1	11.1	<0.01	0.43

†Only data of the fourth harvest (n = 10).

‡KCl-extractable acidity = Exch. Al + Exch. H.

exchangeable-Al content than did the control, while both had similar exchangeable-H content, KCl-extractable acidity, $BaCl_2$-TEA exchange total acidity, and Al saturation. The original pH of the Bh-horizon soil used for all three treatments was 4.6 (Table 6-1). After 139 d under the surface-soil conditions of a grove, the pH of non-amended soil was raised to 5.1 by oxidation and leaching. The pH of the PG was 4.5, and this low pH had a strong effect on soil pH. The high concentrations of soluble salts due to applied PG (Table 6-4) might also have effects on soil pH. Therefore, the pH of the PG-amended soil showed less of an increase than that of the control after 139 d.

The original exchangeable-Al content of the Bh-horizon soil was 1.38 $cmol_c$ kg^{-1} (Table 6-1). After 139 d, the exchangeable-Al content of the non-amended soil decreased to less than 0.68 $cmol_c$ kg^{-1}. The total rainfall of 678 mm occurred on 45 d during the 139-d period (Table A-3). The decrease in exchangeable Al for the control soil thus might have been partially due to leaching. The exchangeable-Al content of the PG-amended soil also decreased after 139 d, though it did not decrease as much as for the non-amended soil. The higher exchangeable-Al content for the PG-amended soil compared to the non-amended soil might be due to the lower pH of the PG-amended soil (Table 6-3). Another source of exchangeable Al for PG-amended soil might have been the Al content of the PG itself, which added another 0.014 cmol Al kg^{-1} of soil.

Highly-significant positive correlations existed between KCl-extractable acidity and exchangeable-Al contents within all three treatments and across all treatments (Table 6-5). Because

Table 6-4. Contrasts of some ions in the saturation extract of soils (four dates).

Ions	Mean (n = 40)			PROB > F	
	Control	Lime	PG	Control vs Lime	Control vs PG
	----- mg kg^{-1} soil ----				
P	0.44	0.32	0.24	0.21	0.05
K^+	2.10	1.66	1.95	0.04	0.47
Ca^{2+}	8.90	15.51	172.35	0.21	<0.01
Mg^{2+}	5.90	3.02	17.46	0.04	<0.01
Na^+	1.62	1.27	1.30	0.17	0.21
Cl^-	2.79	2.61	1.94	0.62	0.03
NO_3^-	1.32	2.25	1.22	<0.01	0.60

Table 6-5. Coefficients of determination (r^2) between acidity and exchangeable Al (four dates).

Acidity	Control (n = 40)	Lime (n = 40)	PG (n = 40)	Total (n =120)
	r^2			
KCl-extractable acidity	0.81^{***}	0.37^{***}	0.49^{***}	0.86^{***}
$BaCl_2$-TEA exchange total acidity	0.29^{***}	NS†	0.30^{***}	0.72^{***}

***Significant at the 0.001 level.

† NS = not significant.

exchangeable-H content was much less than exchangeable-Al content (Table 6-3), KCl-extractable acidity was mainly represented by exchangeable-Al content. Highly-significant positive correlations also occurred between $BaCl_2$-TEA exchange total acidity and exchangeable-Al contents for the control and PG-amended soils.

Some Ions in the Saturation Extracts of Soils

Application of lime did not significantly affect P ($H_2PO_4^-$ and HPO_4^{2-}), Ca^{2+}, Na^+, or Cl^- contents of the saturation extracts, whereas lime significantly reduced K^+ and Mg^{2+} contents and increased NO_3^- content (Table 6-4). The decrease of K^+ and Mg^{2+} contents in lime-amended soil may have been partially due to replacement by Ca^{2+} from the lime, with replaced K^+ and Mg^{2+} then being leached into soil beneath the bags. Dancer et al. (1973) found that increased pH due to liming favored nitrification, which resulted in an increase of NO_3^- content for the lime-amended soil.

Application of PG did not significantly affect K^+, Na^+, or NO_3^- contents of the saturation extracts, though it significantly reduced P ($H_2PO_4^-$ and HPO_4^{2-}) and Cl^- contents, and increased Ca^{2+} and Mg^{2+} contents. The decrease of P ($H_2PO_4^-$ and HPO_4^{2-}) content might be due to the interaction between P ($H_2PO_4^-$ and HPO_4^{2-}) and Al^{3+}, because more Al^{3+} was replaced by Ca^{2+} for the PG-amended soil than for the non-amended soil, although some Al^{3+} might be complexed with F^-. Application of PG resulted in an addition of 25 mg F kg^{-1} of soil. Some Cl^- would be replaced by F^- in the PG-amended soil and subsequently leach into soil beneath the bags. A substantial amount of soluble Ca from the PG applied to the soil resulted in a significant increase in Ca^{2+} of the saturation extract. The Ca^{2+} concentration of PG-

amended soil was much higher than that of the control or lime-amended soil. Much more Mg^{2+} was replaced by Ca^{2+} for the PG-amended soil than for the non-amended and lime-amended soils, and some Mg^{2+} might not have been leached into the soil beneath the bag in the relatively short ensueing period (139 d).

Summary and Conclusions

The objectives of this study were to assess citrus fibrous-root growth in Bh horizon of a Spodosol amended with lime and phosphogypsum (PG), and to measure changes in Al status and other properties of soils due to lime and PG amendments. An implanted soil-mass technique was employed to place some Bh-horizon soil in the surface horizon of a citrus grove. The mixture placed in the mesh bags consisted of Bh-horizon soil of a Smyrna fine sand (Aeric Haplaquods) with an initial pH of 4.6 and amended with either lime (soil pH adjusted to 6.5) or PG (five times the number of equivalents of Al extracted with 1 *M* KCl). Each of the three treatments (control, lime, and PG) was replicated 10 times at each harvest. The bags were removed after 55, 84, 113, and 139 d.

Soil acidity was mainly due to exchangeable Al in the non-amended soil and also in the lime- and PG-amended soils. Application of lime to Bh-horizon soil significantly increased fibrous citrus-root growth. Compared to non-amended soil, the soil amended with lime had higher pH, lower soil acidity, and lower exchangeable Al and Al saturation, as well as higher NO_3^- and lower K^+ and Mg^{2+} contents in the saturation extract. Application of PG to Bh-horizon soil had no significant effect on citrus fibrous-root growth. The soil

amended with PG had lower pH, higher salinity and exchangeable Al; and higher Ca^{2+} and Mg^{2+} and lower P ($H_2PO_4^-$ and HPO_4^{2-}) and Cl^- contents in the saturation extract than the non-amended soil. It seemed that PG-amended Bh-horizon soil after heavy leaching would have increased root growth. However, the probability that citrus groves would adapt to PG-application would be determined largely by long-term effects of PG on soils, citrus yield increase, and economic considerations which were beyond the scope of this project.

CHAPTER VII

OVERALL SUMMARY AND CONCLUSIONS

Aluminum phytotoxicity may be a growth-limiting factor for citrus roots growing in acid soils. Four experiments were conducted in this dissertation to investigate the effects of Al on growth of citrus roots in solution and soil systems. Chapter III discusses the preparation of supernatant nutrient solutions for Al phytotoxicity studies. Chapter IV discusses a nutrient-solution culture experiment conducted in the greenhouse to evaluate the effects of Al on growth of five commonly-grown citrus rootstocks in Florida. Chapter V reports results of a field experiment to evaluate the effects of Al on fibrous root growth in a commercial citrus grove. Chapter VI discusses another field experiment designed to investigate the effects of lime and phosphogypsum on fibrous root growth and chemical properties of Bh-horizon material from a Spodosol.

Two sets of supernatant natrient solutions were prepared, evaluated, and recommended for Al phytotoxicity studies. In the pH 4.0 set and the pH 4.5 set, actual Al concentrations ranged from 0.1 to 171 mg Al L^{-1} and from 0.1 to 10 mg Al L^{-1}, and P concentration was about 1 mg P L^{-1} and 0.2 mg P L^{-1}, respectively. Actual concentrations of Al and P are affected by the preparation procedure.

Five 6-month-old citrus rootstock seedlings were grown in supernatant solution for 60 days. The total new-growth fresh weight might be a better indicator of Al effects on citrus growth than new-growth root length or new-growth shoot height. According to the

response of new-growth fresh weight of whole plants to Al concentration in solution, relative Al-tolerance was Cleopatra mandarin > Rough lemon = Sour orange > Swingle citrumelo > Carrizo citrange. The critical Al concentrations in solution at which toxic effects observed were 12.2, 5.1, 5.1, 4.5, and 1.8 mg Al L^{-1}, respectively. Concentrations below or above the critical Al levels caused either beneficial or toxic effects, respectively. Aluminum concentrations of roots and shoots increased with increased Al concentrations in the growth medium. Aluminum-tolerant rootstocks accumulated more Al in their roots than did the Al-sensitive rootstocks. When Al concentrations in nutrient solution increased from 0.1 to 4.8 mg Al L^{-1}, K, Mg, and P concentrations in roots and K and P levels in shoots increased; whereas Ca, Zn, Cu, Mn, and Fe in roots and Ca, Mg, Cu, and Fe in shoots decreased. It seemed that Al-sensitive rootstocks had lower Cu concentrations in their roots than did Al-tolerant rootstocks at low Al concentration (0.1 mg Al L^{-1}) in solution. The more tolerant rootstocks contained higher Fe concentrations in their roots than did the less tolerant ones when Al concentrations in solution were lower than 8.3 mg Al L^{-1}. Concentrations of the other elements (Ca, K, P, Mg, Zn, and Mn) in roots or shoots appeared to have no certain relationship with the beneficial or toxic effects of Al in nutrient solution, or with Al-tolerance of the rootstocks.

The implanted E horizon of a Spodosol was treated with either lime or four levels of Al and placed in the surface horizon of a citrus grove for 46 days. Results indicated that the critical Al concentration for toxic effects in the saturation extract of soils was 23 mg Al L^{-1} for root growth of 30-yr-old trees of Sour orange

rootstock (C. aurantium L.). This critical value was much higher than that obtained from the solution-culture experiment mentioned above (only 3.9 mg Al L^{-1}). At a concentration of 9.1 mg Al L^{-1}, root-length density was almost twice that of the 0.1 mg Al L^{-1} treatment, and equalled to that of the lime treatment. Aluminum concentrations in root tissues were lower, however, than those in the 0.1 mg Al L^{-1} treatment. Root growth retardation occurred only when the concentration exceeded 23 mg Al L^{-1}. The concentrations of Zn, Fe, and Mn in root tissues decreased with increased Al concentrations in the saturation extract of soils.

Some Bh-horizon soil amended with either lime or phosphogypsum was also implanted in the surface horizon of a citrus grove. The implanted soils were removed after 55, 84, 113, and 139 days. Results showed that application of lime to Bh-horizon soil significantly increased fibrous citrus-root growth. Compared to non-amended soil, the soil amended with lime had higher pH, lower soil acidity, and lower exchangeable Al and Al saturation, as well as higher NO_3^- and lower K^+ and Mg^{2+} contents in the saturation extract. Application of phosphogypsum to Bh-horizon soil had no significant effect on fibrous citrus-root growth. The soil amended with phosphogypsum had lower pH, higher salinity and exchangeable Al; along with higher Ca^{2+} and Mg^{2+} and lower P ($H_2PO_4^-$ and HPO_4^{2-}) and Cl^- contents in the saturation extract than the non-amended soil.

Recommendations for further research include: (1) Evaluation of the relationship between organic-acid contents in citrus roots and shoots and Al-tolerance of citrus rootstocks; (2) Assessment of the physiological functions of citrus roots developed by stimulation of

low Al concentrations in the medium; (3) Determination of mechanisms of beneficial and toxic effects of Al on citrus growth and differential tolerance of citrus rootstocks. A split-root experimental technique may need to be used. The following determinations may also prove helpful: a) root cross-section may be examined using scanning and transmission electron micrographs; b) the deposition of metal ions (Al^{3+}, Fe^{3+}, Mn^{2+}, and Cu^{2+} ions) in selected roots may be assessed using electron microprobe X-ray analysis; c) the rate of cell division may be counted using a photomicroscope; d) the number of binucleate cells may be determined; and e) the CEC and elemental concentrations of roots may be determined; (4) Investigation of effects and causes of spodic horizons on citrus growth with greenhouse studies and field surveys; and (5) Development of methods for predicting Al toxicity to citrus growth in soils.

A P P E N D I X

Table A-1. Initial, final, and new-growth of three parameters for five rootstock seedlings at seven Al levels in solution.

Al concentration in solution	Root length			Shoot height			Fresh weight of whole plants		
	Initial	Final	New-growth	Initial	Final	New-growth	Initial	Final	New-growth
$mg\ L^{-1}$	$cm\ plant^{-1}$						$g\ plant^{-1}$		
				Carrizo citrange					
0.1	131.6±18.9†	750.6±71.1	619.1±66.4	19.8±1.6	35.0±3.4	15.2±2.0	3.8±0.6	13.2±1.7	9.4±1.2
2.7	139.3±11.3	629.3±69.7	490.0±64.5	18.8±1.7	38.1±3.3	19.3±2.7	3.9±0.5	13.4±1.7	9.5±1.2
4.8	134.9±13.1	554.0±70.8	419.0±64.3	17.9±1.6	32.0±2.2	14.1±1.4	3.3±0.3	10.9±1.0	7.6±0.8
8.3	128.8±12.0	507.1±57.1	378.3±57.1	18.8±1.6	28.6±3.0	9.8±1.4	3.7±0.4	9.9±1.4	6.2±1.0
24.4	129.6± 6.1	396.1±50.8	266.5±50.2	18.4±1.8	21.3±2.1	3.0±0.8	3.3±0.5	7.3±1.1	4.0±0.7
28.4	135.4± 8.3	378.7±34.7	243.3±32.2	19.0±1.4	20.3±1.4	1.3±0.4	3.5±0.3	6.9±0.6	3.4±0.4
44.6	136.6±12.0	352.3±29.9	215.7±33.3	18.3±1.5	19.6±1.5	1.2±0.3	3.4±0.6	6.2±0.6	2.8±0.3
				Cleopatra mandarin					
0.1	91.4± 7.2	298.2±32.1	206.8±30.4	14.6±0.7	23.2±1.0	8.7±1.3	2.1±0.2	6.1±0.5	4.0±0.6
2.7	101.7± 8.9	357.0±36.4	255.3±37.4	15.0±1.0	27.3±1.3	12.4±1.5	2.3±0.3	8.0±1.0	5.7±0.8
4.8	98.7± 8.3	387.7±47.6	289.0±45.5	15.3±0.8	27.5±2.1	12.2±1.9	2.5±0.3	8.7±0.7	6.2±0.7
8.3	103.4±11.3	275.7±36.4	172.3±33.2	15.8±0.8	24.4±1.1	8.7±1.5	2.3±0.2	6.6±0.7	4.2±0.6
24.4	104.7±14.9	198.0±29.4	93.3±15.8	14.6±1.0	19.4±2.0	4.8±1.5	2.2±0.4	5.0±1.0	2.8±0.7
28.4	103.0± 9.2	193.8±20.8	90.8±17.5	15.6±0.7	18.4±1.1	2.8±0.7	2.4±0.3	4.7±0.7	2.3±0.5
44.6	106.5±13.1	185.5±22.3	79.0±14.5	15.5±0.8	17.6±1.0	2.1±0.6	2.5±0.3	4.6±0.6	2.2±0.3
				Rough lemon					
0.1	381.0±55.0	948.3±128.7	567.3± 86.0	16.2±1.0	32.5±2.2	16.3±2.0	4.0±0.8	17.1±2.7	13.1±2.0
2.7	420.2±38.9	1199.4±175.5	779.1±150.6	16.7±0.9	36.1±4.3	19.5±4.2	4.7±0.7	24.3±3.9	19.6±3.5
4.8	314.9±53.8	1020.2±119.7	705.3± 92.4	16.7±1.5	35.1±3.0	18.4±2.4	3.9±0.7	19.1±2.3	15.2±1.8
8.3	332.7±97.2	635.8±127.3	303.1± 44.6	15.5±1.3	25.2±3.7	9.7±2.9	3.6±0.9	10.8±3.0	7.2±2.3
24.4	312.3±82.3	466.1±102.1	153.8± 28.6	17.0±1.3	18.4±1.6	1.4±0.3	3.8±1.0	9.0±2.0	5.2±1.1
28.4	398.3±58.4	501.1± 54.2	102.8± 14.2	16.3±0.3	17.9±0.4	1.6±0.2	4.0±0.4	8.4±0.7	4.4±0.5
44.6	311.8±67.8	407.1± 65.2	95.3± 11.7	16.1±1.9	17.7±1.9	1.7±0.5	3.5±0.6	6.5±0.7	3.1±0.4

Table A-1. Continued.

Al concentration in solution	Root length			Shoot height			Fresh weight of whole plants		
	Initial	Final	New-growth	Initial	Final	New-growth	Initial	Final	New-growth
$mg\ L^{-1}$	cm $plant^{-1}$						g $plant^{-1}$		
				Sour orange					
0.1	106.6±15.0	388.2±36.7	287.1±31.3	16.1±1.1	26.1±1.4	10.1±0.9	3.8±0.3	11.2±1.0	7.4±0.8
2.7	109.4±13.6	448.9±83.3	339.6±77.6	15.8±1.5	28.2±1.6	12.4±1.4	3.9±0.5	12.7±1.2	8.9±0.8
4.8	107.1±11.8	412.8±68.2	305.8±61.0	16.5±1.2	28.4±2.0	12.0±1.2	3.8±0.5	12.1±1.2	8.2±0.8
8.3	106.7±13.9	304.5±15.4	197.8±14.0	16.2±1.0	23.9±1.8	7.8±1.3	3.7±0.3	9.5±1.1	5.8±1.0
24.4	116.5±10.3	193.2± 6.4	76.7± 9.3	16.4±0.8	20.0±1.0	3.7±0.8	3.6±0.4	7.4±0.6	3.8±0.5
28.4	112.1±14.6	176.9±20.2	64.8±10.6	15.7±0.6	17.9±0.6	2.2±0.4	3.3±0.4	6.2±1.0	2.9±0.7
44.6	101.9±12.4	167.1± 8.9	65.2±14.6	16.6±0.7	18.1±0.7	1.5±0.5	3.9±0.4	6.3±0.6	2.5±0.4
				Swingle citrumelo					
0.1	203.1±10.8	642.9±74.3	439.8±70.7	21.7±1.4	35.0±1.9	13.4±1.9	6.5±0.5	15.3±1.3	8.9±1.1
2.7	235.9±16.2	748.2±81.8	512.3±69.3	21.8±1.1	37.3±1.6	15.4±2.2	6.6±0.4	17.4±1.3	10.8±1.2
4.8	238.1±22.9	702.2±49.2	465.3±44.2	22.9±1.4	33.8±2.7	10.9±2.5	6.6±0.6	16.2±2.1	9.6±1.8
8.3	217.1±18.4	552.3±45.1	355.2±48.2	22.7±1.6	27.2±2.0	4.4±1.6	6.6±0.5	12.9±1.0	6.3±1.1
24.4	253.7±19.1	473.4±38.5	219.7±32.9	23.1±1.9	26.1±2.7	3.0±1.3	6.8±0.5	12.4±0.8	5.7±0.8
28.4	241.0±16.8	398.9±65.9	182.9±39.8	21.6±1.8	23.5±1.3	1.9±0.7	6.7±0.7	11.3±1.0	4.6±0.4
44.6	273.2±23.1	444.9±36.2	171.7±20.7	21.8±1.1	23.4±1.2	1.6±0.3	6.4±0.5	11.0±1.1	4.7±0.6

† ±Standard deviation (8 replications).

Table A-2a. Elemental contents of roots and shoots of 8-month-old citrus rootstock seedlings after growing in Al-containing nutrient solution for 60 days.

Al concentration in solution	Ca		Mg		K		P	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
$mg\ L^{-1}$	$g\ kg^{-1}$							
				Carrizo citrange				
0.1	3.07±0.05†	6.72±0.11	0.68±0.00	1.28±0.04	11.05±0.39	10.62±0.16	0.81±0.01	0.95±0.02
2.7	2.82±0.05	6.12±0.13	0.96±0.01	1.26±0.03	14.30±0.21	12.75±0.14	2.14±0.06	1.77±0.06
4.8	2.57±0.08	4.77±0.09	0.97±0.02	1.03±0.03	14.37±0.13	11.30±0.08	2.09±0.08	1.72±0.05
8.3	2.37±0.09	3.87±0.11	0.92±0.02	0.90±0.01	11.45±0.18	11.12±0.09	1.67±0.05	1.57±0.05
24.4	2.07±0.05	3.57±0.09	0.90±0.01	0.82±0.01	11.12±0.25	10.12±0.25	1.57±0.05	1.29±0.04
28.4	2.04±0.09	3.69±0.09	1.00±0.03	0.82±0.02	11.35±0.26	10.52±0.29	1.84±0.05	1.29±0.04
44.6	1.99±0.03	3.89±0.06	0.90±0.01	0.86±0.04	9.62±0.20	8.35±0.90	1.39±0.04	1.22±0.03
				Cleopatra mandarin				
0.1	2.92±0.09	8.27±0.20	1.13±0.03	1.43±0.03	13.05±0.17	11.55±0.10	1.14±0.03	0.79±0.01
2.7	2.74±0.04	7.49±0.18	1.71±0.05	1.41±0.05	17.45±0.31	12.95±0.09	2.44±0.09	1.34±0.03
4.8	2.64±0.09	5.87±0.09	1.56±0.08	1.13±0.03	15.50±0.28	12.42±0.17	2.29±0.13	1.42±0.05
8.3	2.49±0.09	4.89±0.18	1.61±0.03	0.92±0.02	12.95±0.34	9.65±0.06	2.14±0.06	1.22±0.05
24.4	2.24±0.11	4.49±0.14	1.43±0.03	0.86±0.02	13.20±0.24	9.55±0.22	2.14±0.06	1.07±0.03
28.4	2.04±0.09	4.87±0.06	0.98±0.04	0.89±0.02	11.62±0.29	8.97±0.16	1.84±0.05	1.09±0.04
44.6	1.99±0.03	4.77±0.06	0.89±0.01	0.89±0.02	9.57±0.25	7.00±0.26	1.39±0.04	1.04±0.03
				Rough lemon				
0.1	3.72±0.03	8.54±0.16	0.83±0.02	1.18±0.04	10.92±0.14	11.20±0.05	1.07±0.05	0.73±0.01
2.7	3.39±0.13	6.72±0.09	1.21±0.05	1.00±0.03	15.10±0.26	11.20±0.17	2.67±0.09	1.74±0.03
4.8	3.24±0.07	4.97±0.14	1.23±0.03	0.94±0.02	12.50±0.15	11.00±0.23	3.02±0.06	1.77±0.05
8.3	3.07±0.08	4.54±0.14	1.11±0.08	1.01±0.03	10.45±0.26	10.37±0.24	2.24±0.06	1.74±0.06
24.4	2.72±0.08	5.34±0.16	0.88±0.09	0.86±0.09	9.10±0.15	9.90±0.23	2.14±0.09	1.29±0.00
28.4	2.64±0.07	5.87±0.11	0.93±0.03	0.81±0.01	9.00±0.15	9.40±0.17	2.12±0.05	1.29±0.04
44.6	2.54±0.06	6.39±0.18	0.90±0.03	0.88±0.01	7.07±0.21	5.62±0.17	2.19±0.04	1.24±0.06

Table A-2a. Continued.

Al concentration in solution	Ca		Mg		K		P	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
mg L^{-1}	----------	----------	----------	g kg^{-1}	----------	----------	----------	----------
				Sour orange				
0.1	4.79±0.10	7.44±0.09	0.82±0.01	1.33±0.03	8.00±0.17	11.17±0.24	0.91±0.01	0.70±0.01
2.7	4.39±0.13	5.47±0.18	1.02±0.04	1.16±0.05	12.70±0.15	12.15±0.09	1.89±0.09	1.24±0.03
4.8	4.24±0.15	5.02±0.05	1.11±0.03	1.11±0.05	11.35±0.21	13.82±0.22	2.12±0.09	1.37±0.05
8.3	3.07±0.06	3.97±0.08	1.01±0.05	0.87±0.02	8.50±0.13	10.45±0.35	1.69±0.04	1.17±0.03
24.4	2.59±0.06	3.64±0.09	0.98±0.04	0.71±0.05	8.37±0.23	8.15±0.14	1.64±0.03	0.97±0.01
28.4	2.39±0.06	3.89±0.06	0.98±0.00	0.76±0.09	7.77±0.12	8.37±0.28	1.54±0.03	1.04±0.03
44.6	2.29±0.06	3.99±0.06	0.98±0.00	0.78±0.06	7.07±0.18	6.40±0.25	1.34±0.05	1.12±0.03
				Swingle citrumelo				
0.1	3.49±0.06	8.64±0.09	1.13±0.03	2.08±0.06	12.45±0.23	10.55±0.09	1.39±0.04	1.09±0.04
2.7	3.22±0.05	7.39±0.06	1.48±0.04	1.98±0.07	14.77±0.21	10.82±0.07	2.54±0.06	1.59±0.04
4.8	3.24±0.09	6.27±0.11	1.68±0.04	1.86±0.06	12.57±0.31	9.95±0.34	2.74±0.10	1.62±0.06
8.3	2.87±0.05	6.09±0.06	1.78±0.04	2.03±0.06	10.12±0.21	8.45±0.15	2.24±0.06	1.62±0.05
24.4	2.57±0.08	5.39±0.12	1.58±0.06	1.83±0.06	9.82±0.18	7.12±0.12	2.07±0.08	1.32±0.05
28.4	2.57±0.05	5.99±0.18	1.66±0.05	1.93±0.06	10.57±0.44	7.32±0.11	2.14±0.06	1.42±0.05
44.6	2.62±0.06	5.52±0.18	1.68±0.04	1.83±0.06	7.50±0.14	5.07±0.12	1.67±0.08	1.24±0.03

† ±Standard error of the mean (4 replications).

Table A-2b. Element contents of roots and shoots of 8-month-old citrus seedlings after growing in Al-containing nutrient solution for 60 days.

Al concentration in solution	Zn		Cu		Mn		Al		Fe	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
$mg\ L^{-1}$	$mg\ kg^{-1}$									
					Carrizo citrange					
0.1	149±1.7†	44±1.1	17±0.9	13±0.9	121±2.8	19±0.4	35±2.8	28±2.5	1190±40.8	145±6.4
2.7	104±2.5	45±1.6	18±0.8	7±0.7	140±3.0	18±0.8	920±24.8	38±2.5	1140±28.9	100±4.1
4.8	81±1.0	38±2.0	18±1.0	8±0.4	94±1.9	18±0.5	938±25.6	38±2.5	920±28.6	130±6.5
8.3	62±3.4	56±1.5	16±0.9	7±0.5	70±1.3	17±0.6	938±17.0	85±6.5	725±23.2	123±6.3
24.4	58±2.2	34±0.4	15±0.7	8±0.6	64±1.7	19±0.6	1640±64.5	90±4.1	713±25.3	125±6.5
28.4	57±1.5	55±2.9	16±0.5	8±0.7	64±1.9	27±0.9	1940±95.7	153±4.8	688±20.2	165±9.6
44.6	48±2.1	49±1.7	18±0.5	9±0.4	48±0.6	29±1.5	2540±104.1	190±9.1	670±14.7	178±11.1
					Cleopatra mandarin					
0.1	160±3.9	43±1.3	27±1.1	7±0.6	120±2.0	28±0.6	43±4.8	35±2.8	1940±64.5	113±4.8
2.7	157±4.3	28±0.5	23±0.5	5±0.3	114±2.1	22±0.5	1390±57.7	58±4.8	1690±40.8	88±2.5
4.8	115±3.1	33±1.0	20±0.9	2±0.3	88±1.5	20±0.8	1520±47.9	45±2.9	1190±0.00	85±2.9
8.3	89±3.0	28±1.3	17±0.4	3±0.3	72±1.7	21±0.7	1590±40.8	110±4.1	1150±47.9	95±5.0
24.4	79±2.1	24±0.7	19±0.6	3±0.4	53±1.8	27±0.6	2220±85.4	113±4.8	780±16.8	105±6.5
28.4	58±1.8	24±0.6	16±0.5	5±0.5	52±1.6	28±1.0	2490±40.8	110±4.1	670±17.8	105±9.6
44.6	48±1.9	28±1.1	18±0.5	8±0.3	47±0.6	30±0.9	2590±40.8	153±6.3	603±13.8	133±6.3
					Rough lemon					
0.1	211±8.5	33±0.5	28±0.8	14±0.6	160±4.1	17±0.5	40±0.0	28±2.5	1990±81.6	90±4.1
2.7	148±6.5	32±1.1	14±0.5	12±0.6	108±2.5	13±0.4	1490±40.8	35±2.9	1870±47.9	70±4.1
4.8	123±4.1	27±0.8	12±0.5	5±0.4	101±3.1	10±0.6	1615±62.9	40±4.1	1370±62.9	55±2.9
8.3	87±1.1	34±1.1	10±0.5	6±0.5	59±1.5	12±0.6	1665±47.9	85±6.5	1120±62.9	55±2.9
24.4	86±1.1	31±0.6	11±0.6	16±0.9	56±1.9	16±0.6	2765±85.4	108±6.3	1000±31.5	68±4.8
28.4	85±1.2	33±0.7	14±0.6	19±1.3	49±1.3	16±0.5	2890±91.3	143±6.3	990±40.8	68±4.8
44.6	83±1.3	29±0.9	12±0.4	41±1.8	39±1.1	17±0.6	6165±165.2	230±7.1	990±40.8	90±7.1

Table A-2b Continued.

Al concentration in solution	Zn		Cu		Mn		Al		Fe	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
mg L^{-1}	mg kg^{-1}									
Sour orange										
0.1	151±4.8	37±1.7	25±0.6	8±0.5	132±0.8	21±0.4	45±6.5	40±4.1	1520±47.9	103±2.5
2.7	111±4.8	35±0.6	19±0.6	7±0.6	82±2.7	20±0.5	950±33.2	45±2.9	1490±57.7	98±4.8
4.8	108±2.9	32±0.9	18±0.5	6±0.4	81±2.1	20±0.6	1003±31.5	68±4.8	1140±28.9	98±4.8
8.3	77±4.4	30±0.6	15±0.4	8±0.5	74±2.0	19±0.5	1090±40.8	113±2.5	1035±32.0	95±5.0
24.4	63±1.6	28±0.6	15±0.5	6±0.3	59±1.1	20±0.6	1990±40.8	115±2.9	815±17.6	120±9.1
28.4	61±1.6	32±0.9	14±0.3	6±0.5	57±2.3	21±0.6	2115±85.4	128±4.8	793±8.5	120±5.8
44.6	58±1.1	32±0.6	16±0.9	8±0.6	56±2.0	21±0.7	2565±85.4	158±4.8	760±14.7	158±6.3
Swingle citrumelo										
0.1	133±4.1	48±1.4	29±0.9	4±0.4	92±2.7	16±0.6	65±2.9	48±4.8	1890±91.3	153±4.8
2.7	89±4.0	44±1.3	20±0.9	4±0.3	47±0.6	12±0.8	1215±47.9	45±2.9	1490±40.8	113±4.8
4.8	70±1.6	46±1.8	16±0.6	5±0.5	43±1.7	12±0.3	1315±25.0	60±4.1	1190±40.8	123±7.5
8.3	54±2.3	59±1.9	13±0.5	4±0.4	37±1.3	10±0.6	1240±64.5	80±4.1	965±47.9	148±7.5
24.4	47±0.4	46±1.7	10±0.5	3±0.3	28±1.0	11±0.9	2165±62.9	103±4.8	610±10.8	145±6.5
28.4	51±3.0	46±1.5	13±0.6	7±0.3	27±1.2	9±0.5	2340±50.0	125±2.9	678±14.9	178±2.5
44.6	41±1.1	38±1.3	10±0.4	4±0.5	24±0.6	7±0.5	3340±132.3	125±2.9	610±28.6	128±2.5

† ±Standard error of the mean (4 replications).

Table A-3. Means and correlations of determination for root length and weight.

Sampling time	Control		Lime treatment		PG treatment	
	Root length	Root dry weight	Root length	Root dry weight	Root length	Root dry weight
	cm bag^{-1}	g bag^{-1}	cm bag^{-1}	g bag^{-1}	cm bag^{-1}	g bag^{-1}
1	1318±277†	0.954±0.191	1075±322	1.037±0.311	729±204	0.604±0.163
2	374±90	0.321±0.074	1234±432	0.920±0.313	520±166	0.509±0.168
3	899±270	0.621±0.193	1248±512	0.927±0.389	1228±491	0.975±0.403
4	881±291	0.675±0.223	1591±796	1.465±0.749	769±323	0.523±0.214
	r^2 = 0.92‡		r^2 = 0.90		r^2 = 0.90	

† ± Standard deviation (10 replications).

‡ All values of r^2 are significant at $P < 0.001$.

Table A-4. Daily maximum and minimum temperatures, and rainfall measurements, at the weather station in Arcadia, Florida (1987).

Day	May		June		July		August		September	
	Temp.	Rainfall	Temp.	Rainfall	Temp.	Rainfall	Temp.	Rainfall	Temp.	Rainfall
	°F	mm	°F	mm	°F	mm	°F	mm	°F	mm
1	86-52		92-70		92-73	15.2	89-71		92-71	41.9
2	88-54		92-70		90-75	2.5	86-72	6.9	87-69	1.8
3	92-55		91-70		91-72		89-72		88-71	14.2
4	90-58		90-71		93-71		90-73		87-71	
5	90-62		91-72		92-73	25.4	93-74		89-72	13.5
6	88-67	18.3	92-70		92-72	33.0	95-73	1.3	88-71	27.4
7	85-68	0.8	85-69	0.8	91-70		95-73		87-73	10.7
8	86-67		86-68		93-70		94-72		90-70	
9	87-73		90-67		92-73		93-74		92-72	12.7
10	87-59	159.5	92-71		93-74		94-73	57.4	91-72	22.9
11	85-64	4.6	91-73	4.6	95-74		88-72	45.7	89-71	11.4
12	87-63	2.5	93-70		94-72		85-73	0.3	89-71	
13	89-62	10.2	92-70		93-74	19.6	78-70	8.4	90-74	0.5
14	89-67		93-72		90-73	1.5	90-70	5.8	89-72	
15	90-67	48.8	95-72		91-75	2.5	92-71		89-72	
16†	88-67		90-72		87-74	6.1	93-70		93-74	
17	89-66	119.4	93-69		91-70	19.8	92-70		94-72	
18	84-66	30.5	92-72		90-71	7.6	92-74		90-71	
19	86-68		93-70		90-72		98-75		93-71	
20	88-68	10.2	94-71	1.3	91-72	17.3	94-73		92-74	

Table A-4. Continued.

Day	May		June		July		August		September	
	Temp.	Rainfall	Temp.	Rainfall	Temp.	Rainfall	Temp.	Rainfall	Temp.	Rainfall
	°F	mm	°F	mm	°F	mm	°F	mm	°F	mm
21	87-67		94-70		90-71	0.8	92-74		90-72	
22	75-65		95-71		90-69		93-73		90-70	1.8
23	90-64		93-71		91-68		92-72		86-72	0.3
24	90-65		95-70		91-74		92-71		86-71	
25	90-67		91-73		93-72		91-71		87-69	
26	89-67		91-74		92-72		90-72		90-69	
27	88-68		85-73		92-71	9.4	92-71		89-68	
28	86-68		84-74		91-72	0.8	91-73		88-73	2.3
29	88-65		90-73		90-73	13.0	90-72		87-71	
30	80-65		80-70		87-71	6.1	92-73		80-73	23.9
31	90-69				90-72	13.2	92-74	6.4		

† For the implanted soil-mass study, the implanting day was May 16, 1987; the four days when samples were removed were July 10, August 8, September 6, and October 1, 1987.

Table A-5. Selected soil properties of three treatments at four sampling times.

Treat-ment	Sampling time	pH	EC_e	KCl-extractable acidity	Exchangeable Al	Exchangeable H	$BaCl_2$-TEA exchange total acidity
			$dS\ m^{-1}$	------------	------------ $cmol_c\ kg^{-1}$	------------	------------
Control	1	5.14±0.21†	0.45±0.05	0.89±0.18	0.73±0.15	0.16±0.03	11.75±0.59
	2	5.32±0.27	0.40±0.05	0.87±0.18	0.68±0.15	0.19±0.04	10.68±0.64
	3	5.22±0.26	0.38±0.05	0.81±0.18	0.64±0.15	0.17±0.04	10.88±0.65
	4	5.11±0.31	0.37±0.06	0.82±0.20	0.66±0.17	0.16±0.05	11.17±0.78
Lime	1	6.65±0.27	0.37±0.06	0.10±0.01	0.10±0.10	0.00±0.00	6.37±0.44
	2	6.32±0.25	0.34±0.06	0.08±0.01	0.07±0.10	0.01±0.00	6.24±0.50
	3	6.23±0.31	0.34±0.07	0.07±0.10	0.07±0.10	0.00±0.00	6.17±0.66
	4	6.26±0.38	0.29±0.05	0.07±0.01	0.07±0.10	0.00±0.00	6.10±0.61
PG	1	4.47±0.22	2.32±0.25	0.96±0.19	0.80±0.17	0.16±0.03	11.61±0.58
	2	4.89±0.29	2.33±0.27	0.95±0.22	0.80±0.18	0.15±0.03	11.37±0.68
	3	5.72±0.30	2.03±0.25	0.88±0.22	0.70±0.18	0.18±0.05	11.26±0.68
	4	4.79±0.29	2.02±0.24	0.92±0.24	0.78±0.22	0.14±0.04	11.52±0.69

† ± Standard deviation (10 replications).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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